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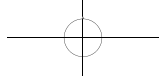
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Lidocaine Regulates the Proliferation and Apoptosis of Colorectal Cancer Cells by Modulating AKR1B1/miR-21-5p Pathway

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Abstract: This study was to investigate the effect of lidocaine on the proliferation and apoptosis of the human colorectal carcinoma cell line (HCT116) and explore the underlying mechanism. HCT116 cells' proliferation and apoptosis rate were determined by CCK8 assay and flow cytometry, followed by treating the cells with 0.5 mM and 1 mM lidocaine. HCT116 cells were transfected with NC-mimic, Mimic-miR-21-5p, inhibitor-NC, and Inhibitor-miR-21-5p, followed by treatment with Lidocaine and fidarestat, combined and both alone. The expression of miR-21-5p and of AKR1B1, PTEN, p-AKT, AKT, and PI3K proteins was determined by qRT-PCR and Western blot. This study find lidocaine inhibited cell proliferation and promoted apoptosis in a time-dose dependent manner. Lidocaine and fidarestat, both alone and in combination, reduced the expression of miR-21-5p and AKR1B1. Lidocaine and fidarestat alone and combined treatments and the miR-21-5p-inhibitor group decreased the expression of p-AKT and PI3K and vice versa in the mimic-miR-21-5p group. The expression of PTEN was increased in the lidocaine + fidarestat group, decreased in the mimic-miR-21-5p group. These results suggest that lidocaine inhibited the proliferation of HCT116 cells and promoted cell apoptosis by downregulating the expression of AKR1B1/miR-21-5p and further modulating the PTEN/AKT/PI3K signaling pathway.

Keywords: Colorectal cancer; Lidocaine; Cell proliferation; Apoptosis; miR-21-5p; AKR1B1

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1. Introduction

Colorectal cancer (CRC) is a common tumor of the digestive tract. Globally, it is the third most common cancer type in males, while it ranks second in females ^[1]. Nowadays, it is a common emerging cancer among the Chinese population ^[2]. Generally, CRC is treated surgically and combined with chemotherapy and targeted treatment ^[3]. However, tumor recurrence and metastasis have been reported in many patients after

CRC surgery, hence minimization of tumor recurrence and metastasis incidents in patients after surgery is a challenging task for effective treatment of CRC. An effective treatment approach could help improve the prognosis and reduce the mortality rate of CRC patients. In clinical practices, lidocaine is a commonly used local anesthetic and antiarrhythmic drug ^[4]. Many studies have shown that lidocaine has an obvious anti-tumor effect and can effectively inhibit the proliferation and invasion of tumor cells, and thus could improve the disease-free survival outcome of tumor patients after surgery ^[5-8].

The occurrence and development of CRC is a complex process of multifactorial, multi-stage, multi-step, and multi-gene changes based on the molecular basis of oncogene activation and tumor suppressor gene inactivation. MicroRNAs (miRNAs) are closely related to tumors and can be used as oncogenes or tumor suppressor genes to regulate the occurrence, development, and metastasis of tumors ^[9, 10]. As a widespread miRNA, aberrant expression of miR-21-5p has been detected in a variety of cancers ^[11]. The human miR-21-5p gene is located in the fragile region of FRA17B on chromosome 17q23.2, which is a fragile site for multiple tumorigenesis. MiR-21-5p can combine with its target gene PTEN, inhibit its expression, widely participate in the regulation of various biological processes of the body, and play an important role as an ‘oncogene’ in tumorigenesis ^[12]. The abundance of miR-21-5p is high, and the changes are stable. Considering the increasing interest in exploring the function and mechanism of miR-21-5p in tumors, we chose miR-21-5p as the research observation index in the present study.

Lidocaine inhibits CRC cell proliferation and promotes cell apoptosis by upregulating the expression of miR-520a-3p ^[13]. Moreover, it inhibits the proliferation, migration, and invasion of lung cancer cells, as well as induces cell apoptosis by downregulating the expression of miR-21-5p ^[14]. However, the mechanism for the regulation of miR-21-5p needs to be further explored. Aldose keto reductase family 1 and member B1 (AKR1B1) ^[15], which catalyzes the reaction of glucose to sorbitol, can be used as a potential marker for tumor detection in lung cancer cells. In various malignant tumors, including CRC, the abnormal expression of AKR1B1 suggests its potential use as a marker for tumor detection as well as a therapeutic agent. Some studies have found that AKR1B1 is overexpressed during the development of CRC, and inhibiting its expression can prevent the growth of CRC cells induced by the growth factors through down-regulation of miR-21 expression ^[16,17]. In this study, we suggested the role of miR-21-5p in the prognosis of CRC; however, its regulatory mechanism induced by CRC needs further exploration.

The present study aims to investigate the effect of lidocaine on the proliferation and apoptosis of the human colorectal carcinoma cell line (HCT116) and explore the underlying mechanism. The cells were treated with lidocaine both alone and in combination with the aldose reductase inhibitor ‘fidarestat’, and their effect on cell proliferation and apoptosis was determined *in vitro*. The effective inhibition of growth and induction of apoptosis of colorectal cancer HCT116 cells by lidocaine indicates its effectiveness in treating CRC.

2. Methods

2.1. Cell Culture

HCT116 cells obtained from Shanghai Zhongqiao Xinzhou Biotechnology Co., Ltd. were used as the study material. Human colorectal carcinoma cells (HCT116) were cultured in DMEM medium (L110, Shanghai BasalMedia Technologies Co., LTD.-Shanghai) containing 10% Fetal Bovine Serum (FBS) (1803122, Biological Industries-Israel) and 1% penicillin/streptomycin (P/S) antibiotics (15070063, Thermo Fisher) and

incubated at 37 °C in a 5% CO₂ incubator (51020241, Thermo Fisher Scientific, USA).

2.2. CCK8 assay

HCT116 cells were digested by trypsin (S330JV, Shanghai BasalMedia Technologies Co., LTD), and a 100 µl solution containing 5×10^3 cells was cultured in a 96-well culture plate and incubated at 37 °C in a 5% CO₂ incubator for 24 h. In parallel, the cells treated with 0 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, and 10 mM lidocaine were incubated for 48 h to determine the effect of different concentrations of lidocaine. Likewise, different concentrations (0 µM, 0.001 µM, 0.01 µM, 0.1 µM, 1 µM, 10 µM, 100 µM, 200 µM, and 500 µM) of fidarestat (HY-105185, MCE) were used to treat HCT116 cells for 48 h to explore the effect of different concentrations. The final volume of each well was maintained at 100 µL, and each drug concentration was set in 3 multiple wells, and the culture plate was incubated at 37 °C. At 48 h, the reaction was stopped and the cell survival rate was determined. The optical density (OD) of the control and treated samples was measured at 450 nm wavelength by using a multifunctional microplate reader (SpectraMax M2e, Molecular Devices, USA).

2.3. Flow cytometry

HCT116 cells were treated with three different concentrations (0, 0.5, and 1 mM) of lidocaine and 100 µM fidarestat and incubated in a 96-well plate for 48 h. After incubation, the cells were digested with trypsin and counted. The cell density was adjusted to 1×10^6 /mL. Thereafter, Annexin V reagent (C1062S, Beyotime, China) and PI were added to the mixture, mixed well, and incubated in the dark for 15 min before analysis *via* flow cytometry by using an inverted fluorescence microscope (OLYMPAS IX71, OLYMPAS, Japan) and FACS Calibur (BD Bioscience, USA).

2.4. Cell transfection

HCT116 cells were resuspended in DMEM medium containing 10% FBS in the absence of antibiotics. The cells were collected and seeded in a 6-well plate at a density of 5×10^4 cells/well, and incubated in a 5% CO₂ incubator at 37 °C for 24 h. After incubation, lipofectamine™ 2000 (11668-019, Invitrogen) was added for transfection, and the liposome 2000/RNA mixture was prepared. Different solutions were prepared as follows: Preparation of solution A: 5 µL miRNA (20 µM) dissolved in 50 µL serum-free 1640 medium; Preparation of solution B: transfer 5 µL liposome 2000 dissolved in 50 µL serum-free 1640 medium, incubate at room temperature for 5 min. Solutions A and B were mixed and placed at room temperature for 20 min. In parallel, 0.2 mL of the original cell culture solution was placed in the culture plate and the mixture of solution A and B was added drop-wise into the cell culture plate. The mixture was incubated in a 5% CO₂ incubator at 37 °C for 48 h. The mixture was replenished with fresh culture medium after sampling for analysis.

Grouping: control, mimic-NC, mimic-miR-21-5p, inhibitor-NC, inhibitor-miR-21-5p, lidocaine (0.5 mM), fidarestat (100 µM), lidocaine (0.5 mM) + fidarestat (100 µM). The miRNA was transfected for 48 h, and the drug acted for 48 h.

2.5. Quantitative real-time polymerase chain reaction

After each treatment, total RNA was extracted from HCT116 cells and the control group by using TRIzol reagent (15596026, Invitrogen, USA). cDNAs were synthesized using ReverTra Ace qPCR RT Kit (FSQ-101, TOYOBO). SYBR Green real-time PCR kit (4368708, Applied Biosystems) was used to carry out

qRT-PCR (ABI7500, ABI, USA). U6 was used as an internal standard to normalize the data. MiR-21-5p: primer sequence (5'-3'): F: TAGCTTATCAGACTGATGTTGAAAA, R: GTGCAGGGTCCGAGGT; U6 F: TTCGTGAAGCGTTCCATATTTT, R: GAATTTGCGTGTTCATCCTTGC. The data was calculated using the $2^{-\Delta\Delta C_t}$ method.

2.6. Western blot

HCT116 cells were lysed with lysis buffer, and the total protein was quantified with the Bicinchoninic acid assay (BCA) kit (Sangon Biotech). The blots were incubated with primary antibody overnight at 4 °C, AKR1B1 antibody (67498-1-Ig, Proteintech), AKT antibody (60203-2-Ig, Proteintech), p-AKT antibody (05-802R, Millipore), PI3K antibody (34050, Cell Signaling), PTEN antibody (22034-1-AP, Proteintech), GAPDH antibody (60004-1-1, Proteintech), HRP-goat anti-mouse IgG (H+L) (A0216, Beyotime biotechnology), and HRP-goat anti-rabbit IgG (H+L) (A0208, Beyotime Biotechnology) antibodies were used. TBST was washed three times, and then the secondary antibody was added and incubated at room temperature for 2 h. TBST was washed three times, and then the PVDF membrane was placed in PBS for 5 min. Thereafter, 200 µL of luminous solution (100 µL A solution + 100 µL B solution) was added for imaging, and then it was placed into the developer for developing the image after 2 min. ImageJ software was used to analyze the gray value of the strip.

2.7. Statistical analysis

The data were expressed as mean \pm standard deviation (SD), and each experiment was performed in triplicate. Statistical software SPSS 22.0 was used for statistical analysis, and the analysis of variance was used for comparing the data among groups. The data was considered statistically significant at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

3. Results

3.1. Inhibition of proliferation of HCT116 cells by lidocaine in a dose-dependent manner

To evaluate the effect of lidocaine on the viability of cancer cells, the HCT116 cells were incubated in the presence of different concentrations of lidocaine for 48 h, and the results are shown in **Figure 1**. According to the cell viability results determined by CCK8 assay, the growth of HCT116 cells was significantly reduced after 48 h, even with 0.5 mM lidocaine treatment, as compared to the control group. Based on these results, the 0.5 mM and 1 mM concentrations of lidocaine and a treatment for 48 h were subsequently selected for determining its effect on the apoptosis of HCT116 cells and a mechanism study through qRT-PCR and Western Blot analysis.

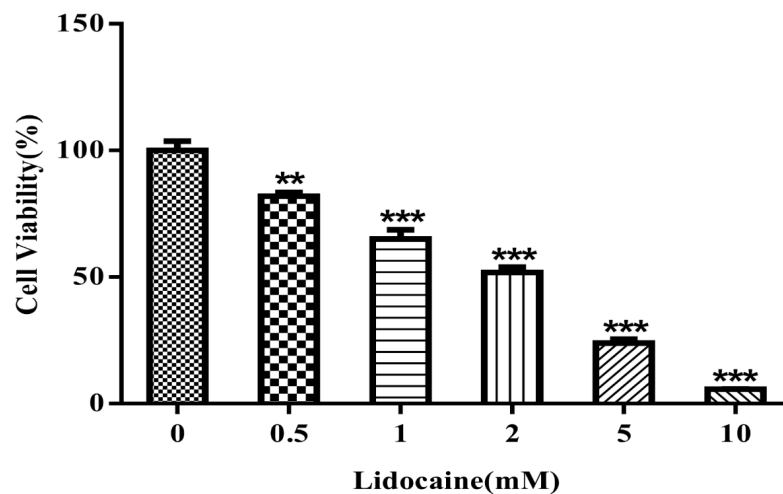


Figure 1. Effect of lidocaine on viability of HCT116 cell proliferation after 48 h. ** $p < 0.01$ vs. 0 mM; *** $p < 0.001$ vs. 0 mM.

3.2. Induction of apoptosis of HCT116 cells by fidarestat

To explore the effect of fidarestat on cell viability, the HCT116 cells were treated with different concentrations (0 μ M, 0.001 μ M, 0.01 μ M, 0.1 μ M, 1 μ M, 10 μ M, 100 μ M, 200 μ M, and 500 μ M) of fidarestat for 48 h. The cell viability analysis of the control and fidarestat-treated cells after 48 h showed that fidarestat significantly reduced the cell proliferation (**Figure 2**). The significant results (** $p < 0.001$) were obtained at 100 μ M and higher concentrations; hence, cells treated with 100 μ M were used for subsequent analysis.

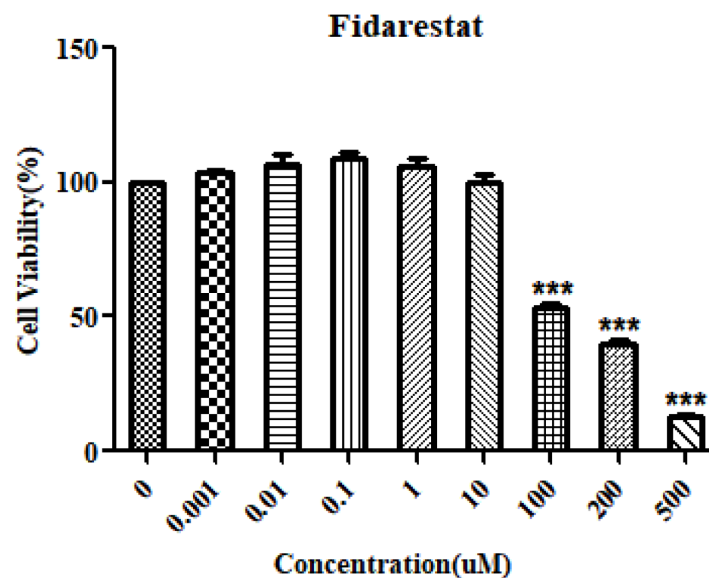


Figure 2. Effect of fidarestat on the proliferation of HCT116 cells after 48 h. *** $p < 0.001$ vs. 0 μ M.

3.3. Lidocaine-induced apoptosis of HCT116 cells

The influence of lidocaine on cell apoptosis was determined by treating the HCT116 cells with 0.5 mM and 1 mM lidocaine for 48 h. The apoptosis rate was calculated based on the cytometry analysis. According to the results, lidocaine not only inhibited cell proliferation but also induced the process of cell death, which led to apoptosis. The highest apoptosis rate was observed upon treatment with 1 mM lidocaine ($*p > 0.05$), while no significant change was observed for 0.5 mM lidocaine treatment (**Figure 3A and B**).

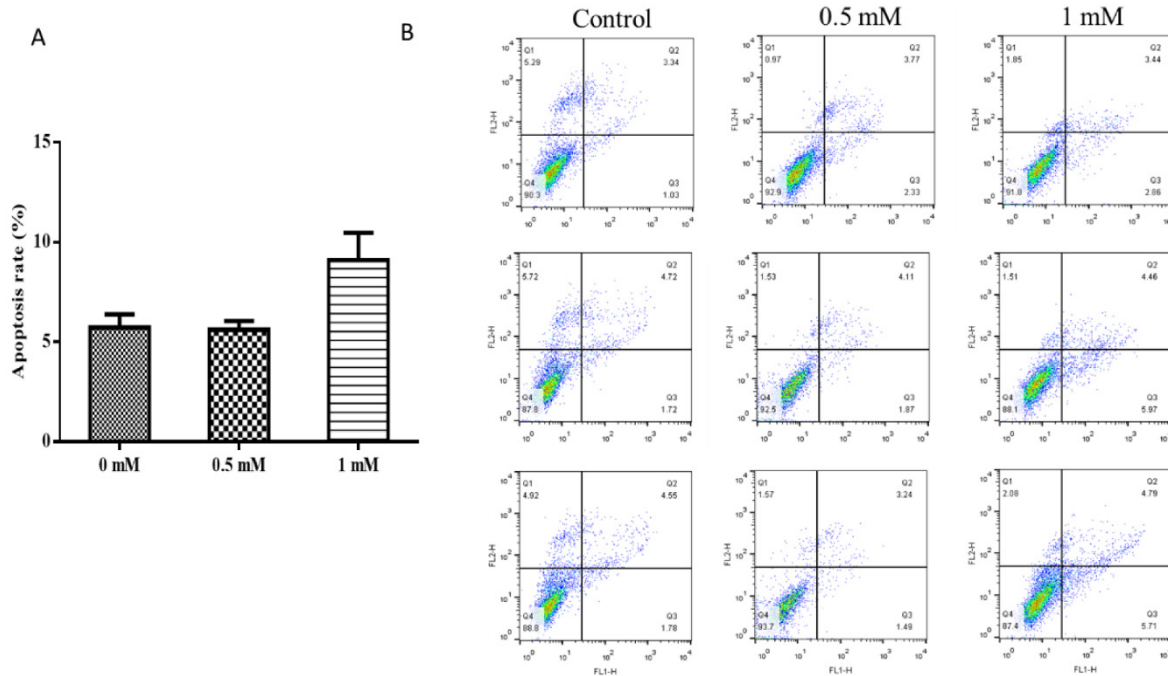


Figure 3. (A) Quantitative and (B) qualitative determination of the effect of lidocaine on apoptosis of HCT116 cells after treatment for 48 h.

3.4. Lidocaine decreased the expression of miR-21-5p in HCT116 cells

To investigate the association between miR-21-5p and lidocaine action on the CRC cells, HCT116 cells were transfected with miR-21-5p mimics and inhibitors, respectively. Compared to the control group, the expression of miR-21-5p in the transfected group was significantly higher ($**p < 0.01$, **Figure 4**), and the expression of miR-21-5p in the miR-21-5p inhibitor group was significantly lower ($***p < 0.001$). Moreover, the expression of miR-21-5p was examined in lidocaine, fidarestat, and combined lidocaine + fidarestat treatment groups. Results showed a decreased expression of miR-21-5p in the lidocaine (0.5 mM) group, and in addition, miR-21-5p expression was significantly decreased in the fidarestat group (100 μ M). The expression of miR-21-5p in the combined lidocaine (0.5 mM) + fidarestat (100 μ M) group was also significantly decreased ($***p < 0.001$), suggesting that miR-21-5p may play an important role in CRC cell proliferation.

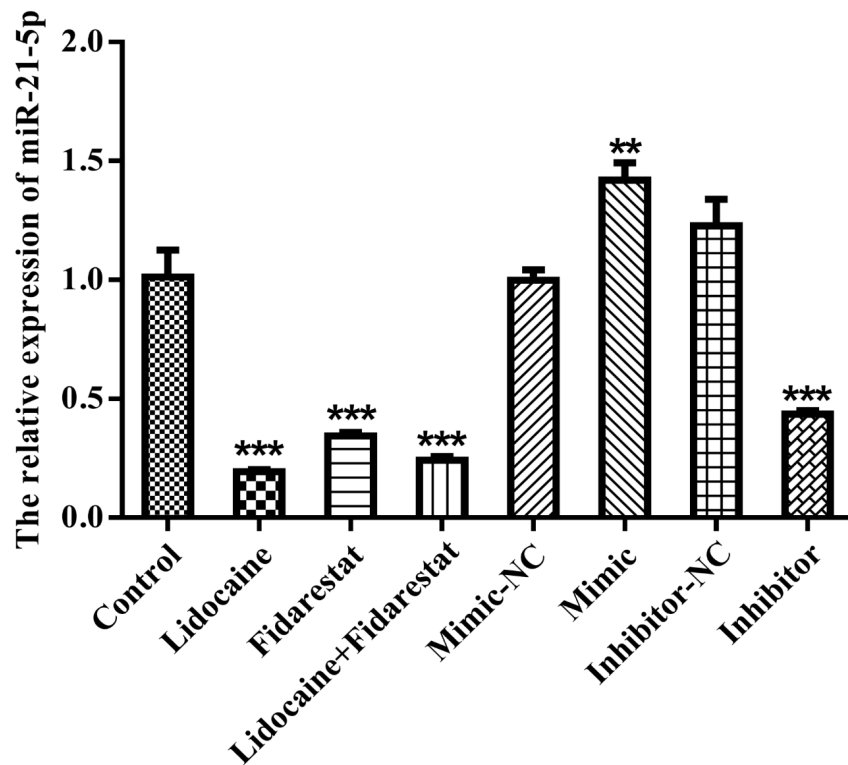


Figure 4. Expression level of miR-21-5p detected by qRT-PCR. ** $p < 0.01$ vs. Control; *** $p < 0.001$ vs. Control.

3.5. Lidocaine decreases the expression of AKR1B1 and regulates the PTEN-PI3K-AKT signaling pathway in CRC

To investigate the effect of lidocaine, fidarestat, and miR-21-5p mimic on the expression of AKR1B1, AKT, p-AKT, PI3K, and PTEN proteins, their expressions were measured through Western blot (**Figure 5A**). The relative expression of all proteins was analyzed and presented in **Figure 5B–F**. The expression results show that the expression of AKR1B1 was significantly decreased after lidocaine and fidarestat treatment. The decreased expression after both alone and combined lidocaine + fidarestat treatment was also noticed (** $p < 0.01$ or *** $p < 0.001$) (**Figure 5B**). Whereas no significant change in the expression level of AKR1B1 was noticed in miR-21 mimic and inhibitor-transfected cells ($p > 0.05$) compared to the control. The results did not show a significant difference in the expression of AKT in both the mimic and inhibitor groups of miR-21-5p. A slight increase in the mimic group, while a minor decrease in the inhibitor group was noticed as compared to their respective control groups (**Figure 5C**). The expression of p-AKT (**Figure 5D**) and PI3K (**Figure 5E**) was significantly increased (** $p < 0.001$) in the mimic-miR-21-5p group, and low values were obtained after lidocaine and fidarestat alone and combined treatments (** $p < 0.001$). The low expression of p-AKT and PI3K was also recorded in miR-21-5p inhibitor-transfected cells. The mimic-transfected cells showed low expression of PTEN, while cells transfected with miR-21-5p inhibitor showed high PTEN expression compared to the control group (**Figure 5F**). The high expression of PTEN was noticed after lidocaine and fidarestat combined treatment. These results suggest a major role of miR-21-5p in AKR1B1 expression and regulation of the PTEN-PI3K-AKT signaling pathway in CRC.

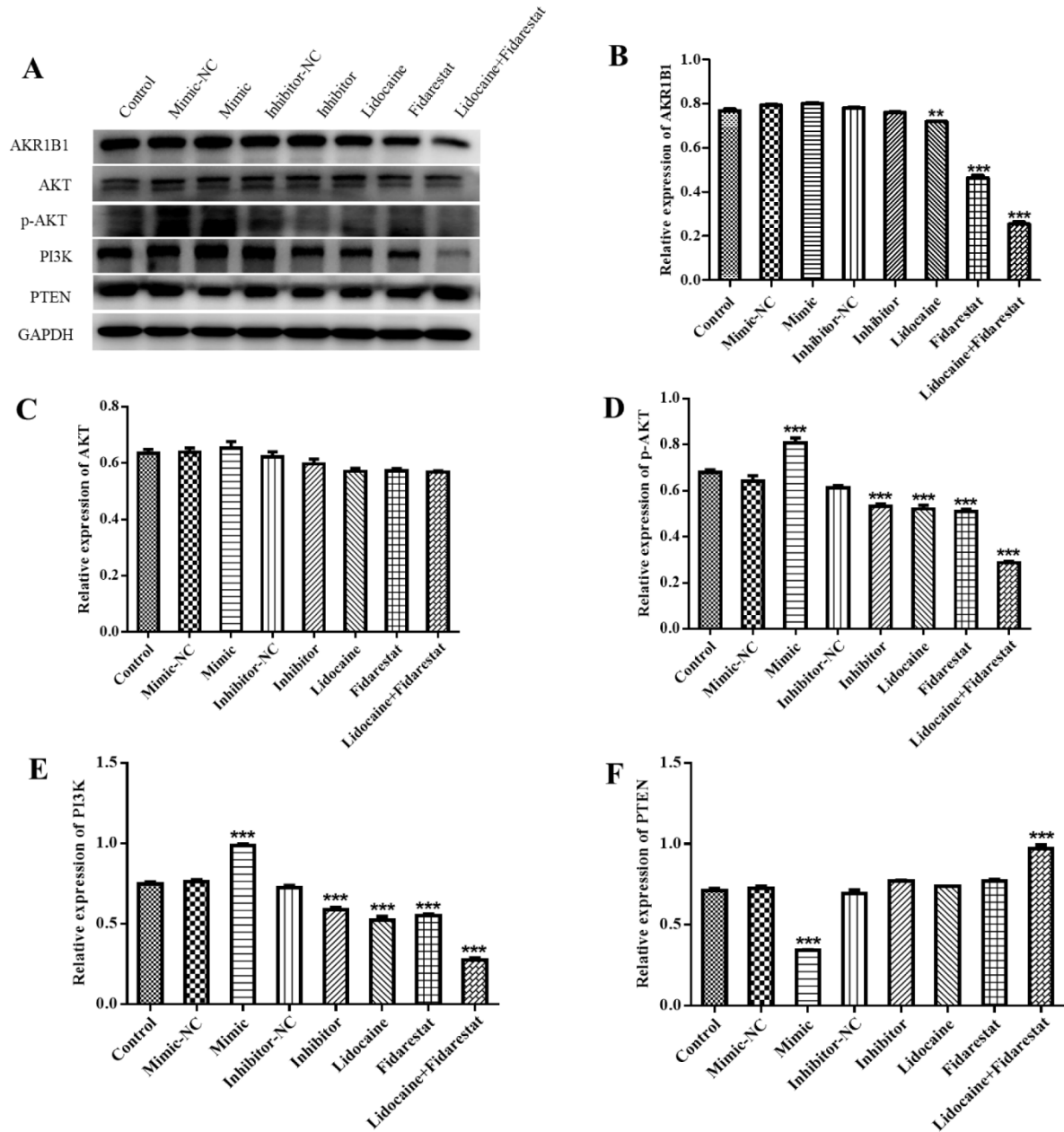


Figure 5. Western blot analysis of protein expression level.(A) Western Blot analysis, (B) AKR1B1 gray quantification, (C) AKT quantification, (D) P-AKT gray quantification, (E) PI3K gray quantification, and (F) PTEN gray quantification. ** $p < 0.01$ vs. Control; *** $p < 0.001$ vs. Control

4. Discussion

In the present study, lidocaine treatment effectively inhibited the proliferation of HCT116 cells in a dose-dependent manner, with 0.5 mM and 0.1 mM lidocaine found effective for inhibiting cell proliferation. Moreover, lidocaine promoted cell apoptosis by downregulating the expression of AKR1B1/miR-21-5p and

further modulated the PTEN/AKT/PI3K signaling pathway. Interestingly, a combined treatment of HCT116 cells with lidocaine and fidarestat showed a synergistic apoptotic effect, indicating their usefulness in treating CRC.

Lidocaine tends to inhibit cancerous cell growth and spread, especially in CRC. The findings of the present study are consistent with previous studies that showed the negative effect of lidocaine on lung cancer cells' viability by downregulating the expression of miR-21-5p^[14]. Another study suggested that lidocaine upregulated the expression of miR-520a-3p and inhibited CRC cell proliferation and induced cell death^[13]. Lidocaine also controls the miR-1204 expression and prevents CRC occurrence^[18]. This study reports the effect of lidocaine on miR-21-5p expression in CRC. Lidocaine significantly inhibited the expression of miR-21-5p and the expression of PI3K, p-Akt proteins. It was found that the apoptosis of HCT116 cells was significantly increased after 1 mM lidocaine treatment; however, there was no statistical significance, which may be related to the relatively small concentration of lidocaine used. Studies have shown that the concentration of lidocaine in promoting apoptosis in gastric cancer^[19,20], breast cancer^[21], lung cancer^[22], and other cancer cells was higher than 1 mM^[23,24]. Our findings validate the involvement of miR-21-5p in regulating the downstream PTEN/PI3K/AKT signaling pathway, which was previously identified by Yang et al.^[25]. This signaling network is critical for CRC progression and prognosis. This study found that after HCT116 cells were transfected with mimic-miR-21-5p, the expression level of PTEN was significantly reduced, and the expression level of PI3K and p-AKT was significantly increased. However, after treatment with miR-21-5p inhibitors, the expression level of PTEN did not change much, and the expression of p-AKT and PI3K was significantly decreased. The changing trend was basically consistent with that of Yang et al.^[25]

AKR1B1 is an NADPH-dependent monomer enzyme and also a rate-limiting enzyme in the polyol pathway of glucose metabolism^[26]. It has been widely studied due to its important role in the occurrence and development of diabetes complications and various oxidative stress diseases^[26]. In recent years, studies have found that AKR1B1 was overexpressed in various forms of malignant tumors^[27,28], which can lead to cancer cell metastasis, adhesion, invasion, and migration^[29]. It has been reported that inhibition of aldose reductase can prevent the growth of colon cancer cells induced by the growth factors by down-regulating the expression of miR-21 expression^[17]. These studies suggested that aldose reductase may be a potential tumor marker and a new therapeutic target for CRC. In addition, the aldose reductase inhibitor, fidarestat, can improve the sensitivity of doxorubicin to CRC cells and reduce its cardiotoxicity^[30]. This study found that both fidarestat and lidocaine can inhibit the proliferation of CRC cells and reduce the expression level of microRNA-21. The expression level of PTEN increased while that of PI3K and p-AKT decreased after a combination of lidocaine and fidarestat. These results indicate that there were similarities in the anti-tumor mechanism between lidocaine and fidarestat in CRC. Moreover, this study also found that lidocaine inhibited the expression of AKR1B1, suggesting that the anti-tumor effect of lidocaine may be related to the inhibition of aldose reductase, and its specific mechanism needs to be further explored.

5. Conclusion

In summary, this study showed that lidocaine inhibits the proliferation of HCT116 and promotes its apoptosis by downregulating AKR1B1/miR-21-5p, which ultimately controls PTEN/PI3K/AKT expression in CRC. This study provides an experimental basis for the application of lidocaine in the perioperative period of CRC, and also provides a new reference for lidocaine to become an ideal anesthetic in the surgical treatment

of CRC, to improve the prognosis of CRC patients. The impact of lidocaine on aldo reductase-miR-21-5p expression and related pathway was identified for the first time in CRC. However, there is still a need to identify other target elements and regulating factors that are involved downstream in this pathway. The identification of the regulatory network associated with miR-21-5p can be further carried out in future studies to better understand the underlying mechanism.

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Disclosure statement

The authors declare no conflict of interest.

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Mapping and Visualization of Global Research on Bispecific Antibodies in Solid Tumors: A Bibliometric Analysis (2006–2025)

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Abstract: Background: Although bispecific antibodies (BsAbs) hold promising therapeutic prospects in solid tumors, there is scarce bibliometric analysis on this field. This study aimed to systematically map the research landscape, identify key trends, and highlight future directions for BsAb research in solid tumors. Methods: Literature of BsAb development for solid tumors was retrieved from Web of Science Core Collection published between 2006 and 2025. Publication distribution, research trends and hotspots were analyzed and visualized by VOSviewer, CiteSpace and the R package “bibliometrix”. Results: Overall, we identified 3632 publications stemming from a fast-growing field currently strongly influenced by the United States and China, with Memorial Sloan Kettering Cancer Center and National Cancer Institute the most productive research centers, Front Immunol the most productive journal and J Clin Oncol the most cited. The dominant research topics that appeared from keyword analysis are bispecific antibody, immunotherapy, targeted therapy, non-small cell lung cancer, and EGFR. Conclusion: This study documents the evolution of BsAb research, from basic science to clinical translational research in solid tumors. The research in this field is highly mature at this point, with emphasis on the targeting of specific tumor types and specific antigens. In order to further push forward the frontier, greater international and subfield collaborations, as well as larger-scale clinical trials targeting broader indications, overcoming resistant mechanisms and combination strategies are necessary.

Keywords: Bibliometric analysis; Bispecific antibody; Solid tumors; Immunotherapy; Targeted therapy

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1. Introduction

Although approaches for treating solid tumors have advanced dramatically in the past decades, there still exist significant challenges. Existing systemic therapeutic methods such as chemotherapy, targeted therapy, and immune-based therapy are still likely to yield unsatisfying efficacies, severe side effects and primary

or acquired resistance ^[1–3], which demands innovative therapeutic approaches. The recent advancement of Bispecific antibodies (BsAbs) represents a promising avenue to overcome these drawbacks.

The concept of BsAbs was first suggested by Nisonoff and his colleagues at the beginning of the 1960s ^[4], but the technological realization faced significant difficulties ^[5]. The first generation of BsAbs was produced either by chemical linking or by hybrid-hybridoma (quadroma) technology, which suffered a lack of stability, low titer and high immunogenicity ^[6,7]. The key advance was with the introduction of developments in molecular engineering through genetic engineering, which allows the accurate pairing of heavy and light chains of an antibody, thus forming a stable bispecific structure ^[8,9]. In recent decades, increasing research has focused on the development of BsAb for solid tumors ^[10]. BsAbs are genetically engineered proteins that bind two different antigens or two epitopes on the same antigen sequentially or simultaneously and thus can potentially initiate mechanisms beyond the simple additive activity of two mAbs ^[11]. Compared to mAbs, BsAbs offer the advantage of enhanced targeting with dual specificity, resulting in higher binding efficiency, reduced susceptibility to resistance, and unique functions, including direct engagement of T cells to selectively kill tumor cells ^[12]. Meanwhile, the single antibody architecture of BsAbs means less Fc fragment exposure, thus reducing their adverse effects.

BsAbs have changed the treatment paradigms of hematological malignancies in the past decade ^[13,14] with the approval of T-cell engager blinatumomab for acute lymphoblastic leukemia ^[15]. Nevertheless, their clinical use in solid tumors is rather limited due to several confounding factors: the immunosuppressive tumor microenvironment (TME) inhibiting T-cell activity, low density of tumor-antigens combined with their expression in normal tissues (on-target/off-tumor effects), difficulty of drug penetration caused by stromal barriers, and resistance mechanisms caused by the loss of antigens ^[16]. Nevertheless, rapid progress has been achieved. As of October 2025, eight BsAbs have been approved to treat solid tumors and four of them were approved in 2024 ^[2,17–19], which indicates a recent blossom in clinical success.

Although BsAbs feature attractive characteristics for their roles in treating solid tumors, there are unanswered questions regarding the mechanisms involved in their boost of immune system activity against solid tumors, as well as the resolutions to on-target/off-tumor effects. Furthermore, the trajectory and the research hotspot of BsAbs in solid tumors have not yet been analyzed by bibliometric analysis. Based on these, the purpose of our study is to analyze the research and development of BsAbs in solid tumors and to describe research hotspots, keywords, and collaborators in this field. This study was conducted based on bibliometric analysis, a method that enables us to quantitatively evaluate scientific papers ^[20] through standard data collecting and mining from publications, citations and research collaborations on BsAbs and solid tumors. We aim to provide a comprehensive overview of research in this field, as insights may emerge that guide forthcoming research and clinical practice.

2. Materials and methods

2.1. Data source and search strategy

The literature search was conducted in the Web of Science Core Collection (WoSCC). The searching time frame was around 20 years (January 1, 2006–November 7, 2025). The whole process of data extraction and export was performed in one day (November 7, 2025) to reduce possible discrepancies due to daily database updates. The search terms were as follows: TS = (*cancer* OR *neoplas* OR *tumo* OR *carcinoma* OR *adenocarcinoma* OR *metasta* OR *malignan* OR *sarcoma* OR *melanoma*

OR *oncolog*) AND TS = (bispecific antibod* OR bispecific monoclonal antibod* OR bsab* OR bifunctional antibod* OR bifunctional monoclonal antibod* OR T-cell engager* OR T cell engager* OR Catumaxomab OR Amivantamab OR Tebentafusp OR Cadonilimab OR Tarlatamab OR Itonescimab OR Zanidatamab OR Zenocutuzumab) NOT TS = (leukemia* OR leukaemia* OR lymphoma* OR myeloma* OR myeloproliferative disorder* OR hematologic* OR haematologic* OR blood cancer*). The inclusion of publications was restricted to articles and reviews published in English. The literature search and screening processes are shown in **Figure 1**.

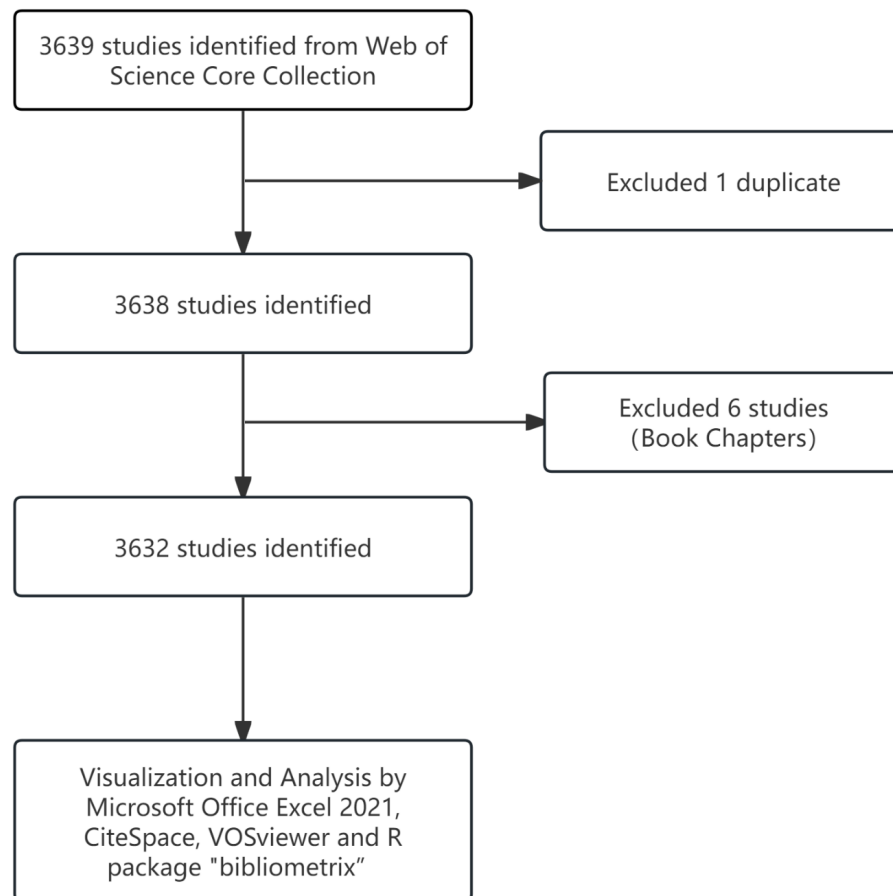


Figure 1. Flowchart illustrating the search strategy and selection process.

2.2. Bibliometric analysis and visualization

The bibliometric analysis was performed using VOSviewer (version 1.6.20), CiteSpace (version 6.2.R3), and the R package “bibliometrix” (version 4.2.1). VOSviewer was used to generate collaboration, co-citation, and co-occurrence networks for countries, institutions, authors, journals, and keywords. In the generated maps, the size of a node corresponds to a metric (e.g., publication count), its color denotes a cluster classification, and the thickness of the connecting lines indicates the strength of the relationship (e.g., collaboration or co-citation frequency). CiteSpace was used to generate a dual-map overlay of journals and to identify references and keywords with citation bursts. The “bibliometrix” package (<https://www.bibliometrix.org>) was used for

the analysis of thematic evolution and the construction of a global publication distribution network. Journal metrics, including the quartile and impact factor, were obtained from Journal Citation Reports (JCR) 2024. Microsoft Office Excel 2021 was used for quantitative analysis of annual publication trends.

3. Results

3.1. Annual publication trend

The study obtained 3632 publications in total from the database, containing 2600 articles and 1032 reviews. The number of annual publications showed an upward trend, increasing from 32 in 2006 to more than 100 in 2015 (**Figure 2**). Following a period of gradual growth, a sharp increase occurred in 2019, after which the publication counts rose dramatically to a peak of 604 in 2025. The growth curve was well fitted with a polynomial ($R^2 = 0.9712$) in Microsoft Office Excel 2021, which verified this positive correlation between the publication year and output. This quantitative assessment confirms a significant trend statistically and illustrates a growing interest in BsAbs for solid tumors over recent years.

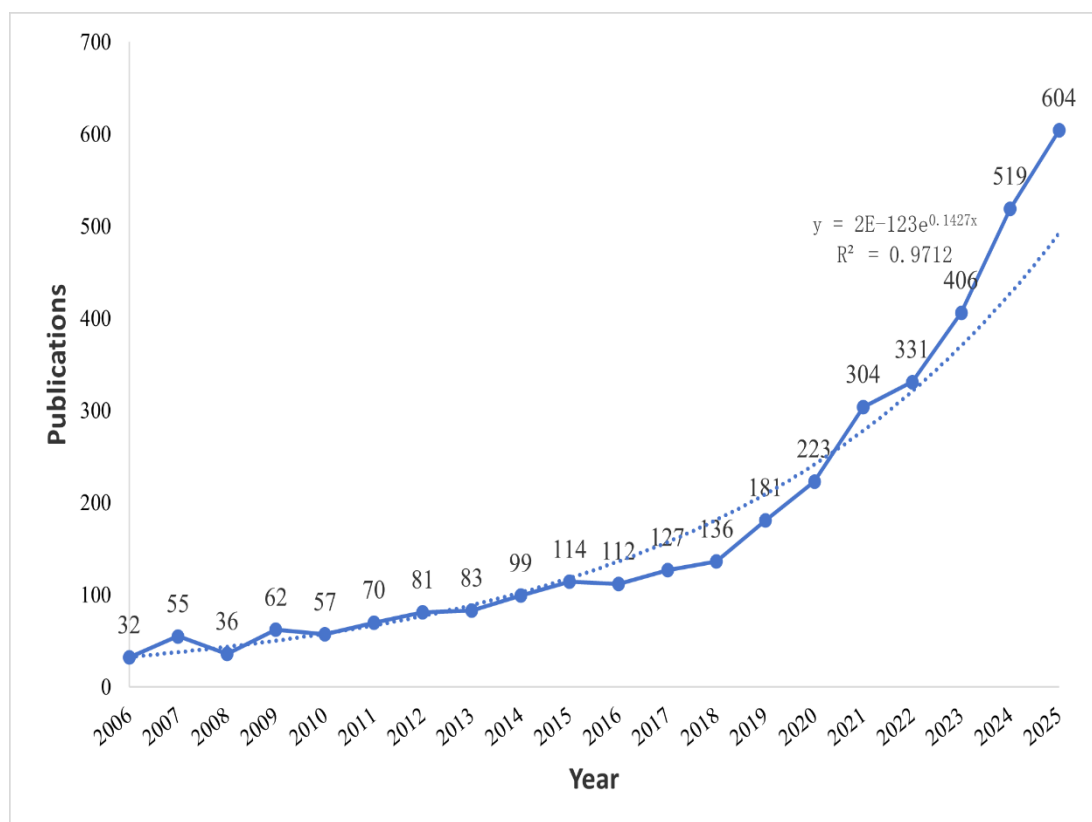


Figure 2. Annual output of bispecific antibody research for solid tumors.

3.2. International contributions by country/region and institution analyses

Publications originated from 78 countries/regions and 4375 institutions. The top 10 countries/regions are from Asia, Europe and North America, mainly Europe ($n = 6$) and Asia ($n = 3$) (**Table 1**). The United States (US) was the biggest contributor (1400 publications, 38.5%), followed by China (903, 24.9%), Germany (511, 14.1%) and the United Kingdom (229, 6.3%). Notably, the US and China account for 63.4% of the total publications. The collaborative network was generated according to the publication amount and inter-country

collaborations, with a threshold of a minimum of 5 publications per country/region (**Figure 3A**). The network includes a total of 51 countries/ regions, which shows a high degree of international cooperation (**Figure 3B**). For instance, the US has been working closely with nations like China, Germany, and South Korea. China's cooperative engagements, though globally extensive, are relatively less intensive.

Table 1. Top 10 countries and institutions in bispecific antibody research for solid tumors

Country	Counts	Institution	Counts
The US	1400	Memorial Sloan Kettering Cancer Center (The US)	102
China	903	National Cancer Institute (The US)	61
Germany	511	Sun Yat-Sen University (China)	58
The United Kingdom	229	Huazhong University of Science and Technology (China)	57
France	201	The University of Texas MD Anderson Cancer Center (The US)	56
Japan	197	Zhejiang University (China)	54
Switzerland	184	Chinese Academy of Sciences (China)	52
Netherlands	168	Harvard Medical School (The US)	50
South Korea	165	German Cancer Research Center (Germany)	50
Italy	164	Shanghai Jiao Tong University (China)	49

Country Collaboration Map

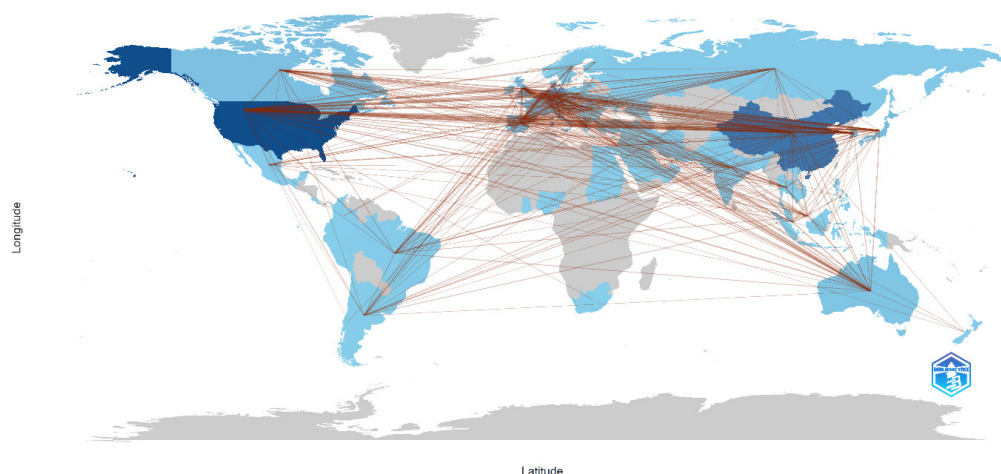


Figure 3A. Geographical distribution of bispecific antibody research in solid tumors.

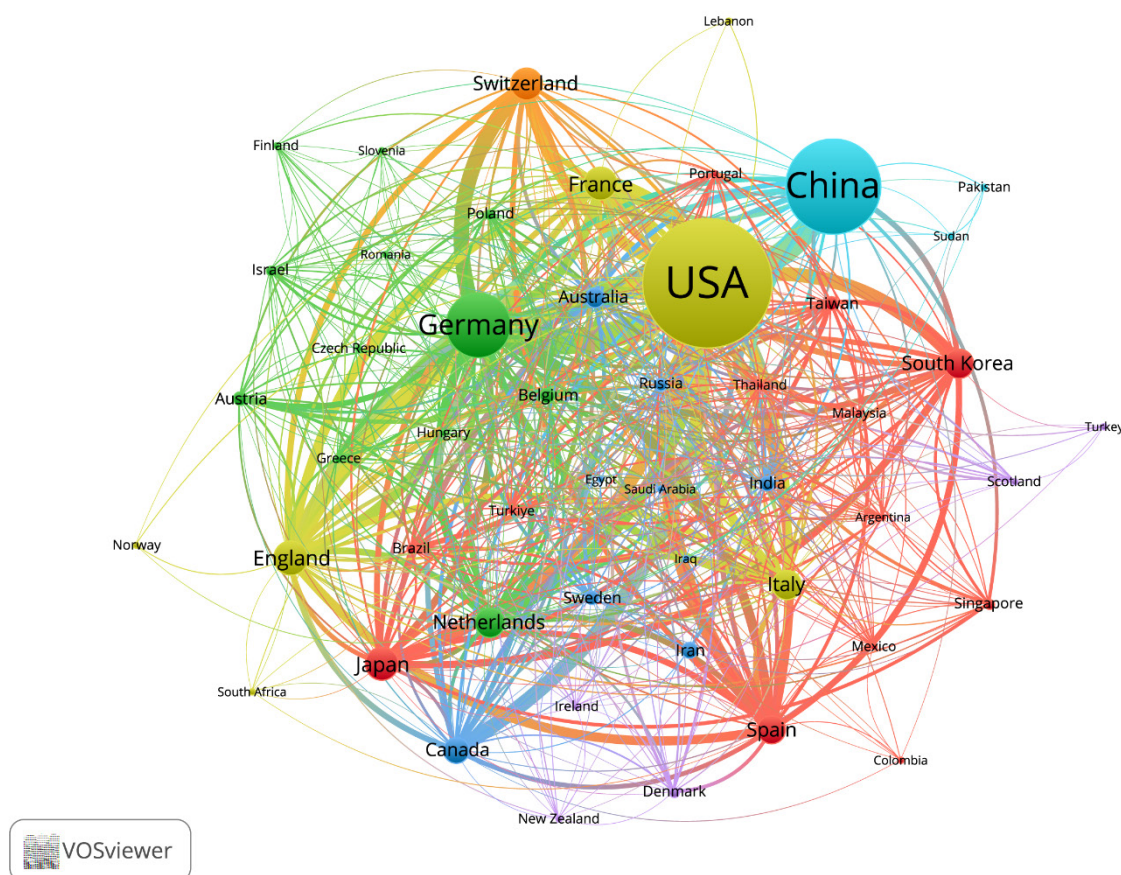


Figure 3B. Visualization of countries on bispecific antibody research in solid tumors.

Among the top 10 institutions, the majority were distributed between two countries, with nine located in either China or the US. Memorial Sloan Kettering Cancer Center ($n = 102$, 2.8%), National Cancer Institute ($n = 61$, 1.7%), and Sun Yat-sen University ($n = 58$, 1.6%) were the top three institutions in terms of the number of relevant publications. We selected a subset of 48 institutions, with a minimum of three publications per institution, for further analysis. A collaborative network was then built to visualize the publication relationships and number of co-authorships among institutions (**Figure 4**). The figure clearly shows that institutions have extensive cooperation, with Memorial Sloan Kettering Cancer Center and MD Anderson Cancer Center at the core of the collaborative network. In addition, solid collaborations exist between academic, clinical and industry institutions (Genentech, Amgen, Roche Innovation Center), pushing BsAb research forward in both fundamental science and clinical practice.

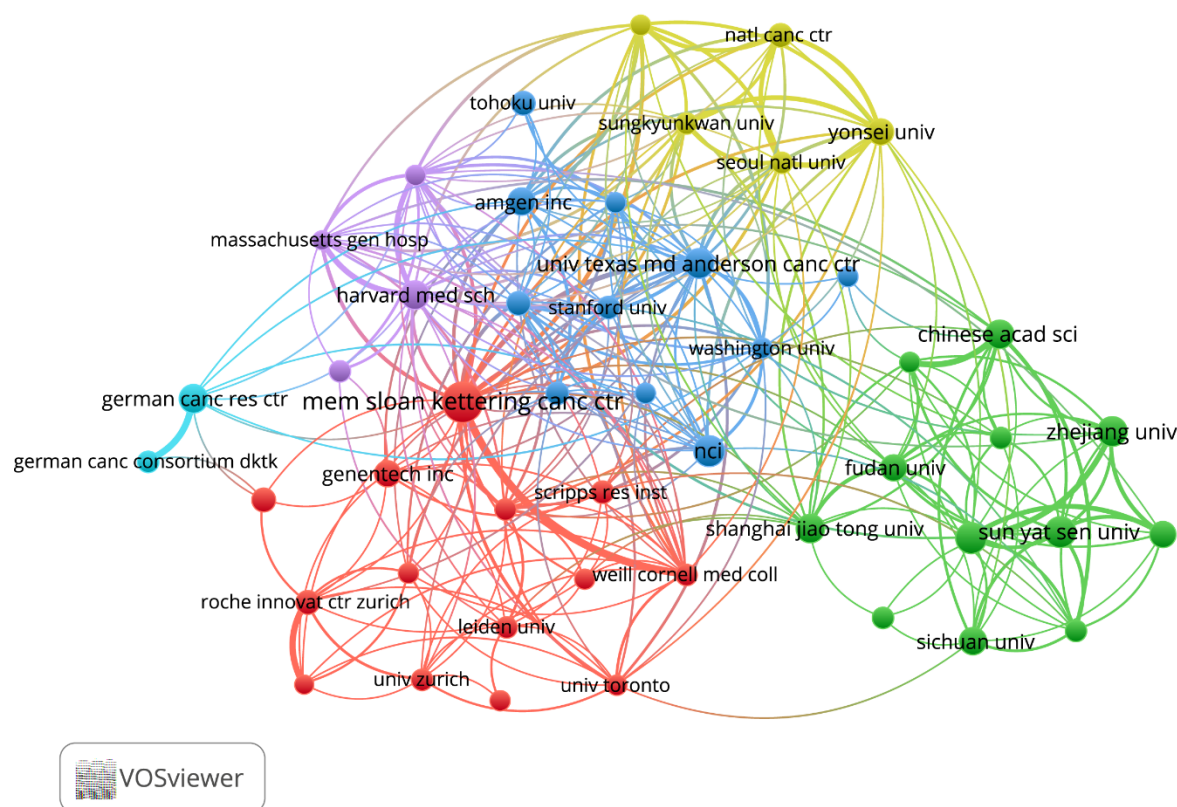


Figure 4. Visualization of institutions on bispecific antibody research in solid tumors.

3.3. Journals and co-cited journals

An analysis of the scholarly landscape identified 836 journals publishing on BsAbs research in solid tumors. The top 10 journals and the most cited journals are listed in **Table 2**. The influence of the journals was evaluated based on the most recent Journal Impact Factor (IF) and JCR quartile retrieved from WoSCC. The leading journal in terms of the number of relevant publications was *Front Immunol* (n = 137, 16.4%), followed by *MAbs* (n = 115, 13.8%), and *Cancers (Basel)* (n = 83, 9.9%). Of the top 10 most productive journals, *Clin Cancer Res* (IF = 10.2), *MAbs* (IF = 7.3), and *Oncoimmunology* (IF = 6.3) had the highest IF according to JCR 2024. The journal co-occurrence network was mapped for 48 journals that published a minimum of 14 relevant articles (**Figure 5A**). This network illustrates active citation relationships, notably positioning *Front Immunol* as a central hub connected to journals such as *MAbs* and *J Immunother Cancer*.

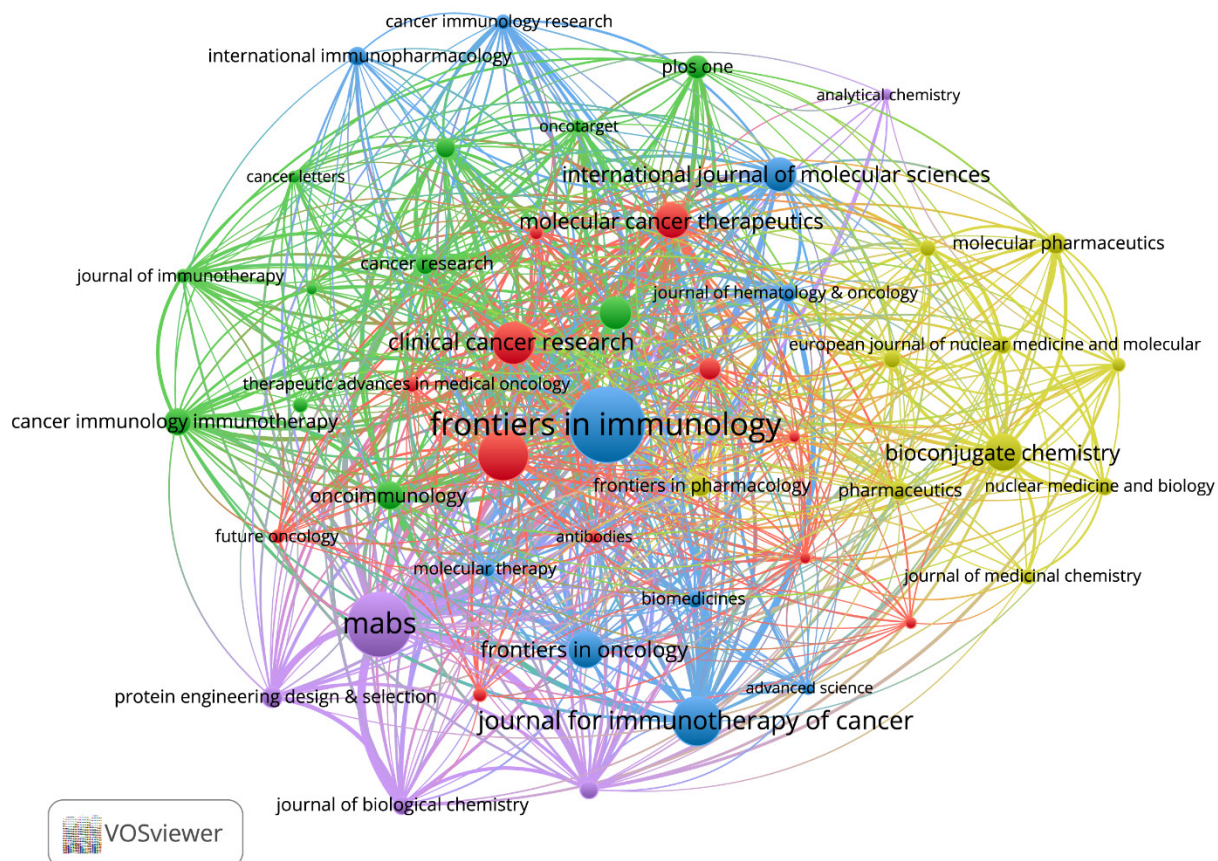


Figure 5A. Visualization of journals on bispecific antibody research in solid tumors.

As shown in **Table 2**, all of top 10 co-cited journals were co-cited over 3000 times, while the top three were *J Clin Oncol* (8998), *Clin Cancer Res* (7698), and *Cancer Res* (6823). Most of these journals were highly influential, as 90% of them were ranked in the JCR Q1 quartile and 60% with IF > 10, led by *N Engl J Med* (IF = 78.5), *Ann Oncol* (IF = 65.4), and *Nature* (IF = 48.5). Figure 5B displays the co-citation network, in which journals that have been co-cited over 480 times are included. The analysis demonstrates a highly integrated landscape of BsAb research. Among the three clusters, journals in the green cluster primarily focus on fundamental research, such as tumor biology and biotechnology, aiming to investigate the mechanisms of BsAbs and drug development. Journals in the blue cluster, predominantly clinical oncology publications, serve to synthesize data from landmark clinical trials and provide clinical validation for the research. The red cluster consists of journals focused on cancer immunology and immunotherapy, bridging the fundamental immunology with the application of BsAbs in solid tumor treatment. This visualization shows that *J Clin Oncol* demonstrates significant co-citation, frequently co-cited with journals such as *Clin Cancer Res*, *N Engl J Med* and *Ann Oncol*.

Table 2. Top 10 journals and co-cited journals on bispecific antibody research in solid tumors

Journal	Count	IF (2024)	JCR (2024)	Journals	Total Citations	IF (2024)	JCR (2024)
Front Immunol	137	5.9	Q1	J Clin Oncol	8998	41.9	Q1
MAbs	115	7.3	Q1	Clin Cancer Res	7698	10.2	Q1
Cancers (Basel)	83	4.4	Q2	Cancer Res	6823	16.1	Q1
Clin Cancer Res	67	10.2	Q1	N Engl J Med	5320	78.5	Q1
Bioconjug Chem	57	3.9	Q1	Proc Natl Acad Sci U S A	4713	9.1	Q1
Front Oncol	56	3.3	Q2	Nature	3563	48.5	Q1
Mol Cancer Ther	53	5.5	Q1	Ann Oncol	3545	65.4	Q1
Int J Mol Sci	51	4.9	Q1	Front Oncol	3294	5.9	Q1
Sci Rep	50	3.9	Q1	Mabs	3152	7.3	Q1
Oncoimmunology	40	6.3	Q1	J Immunol	3149	3.4	Q2

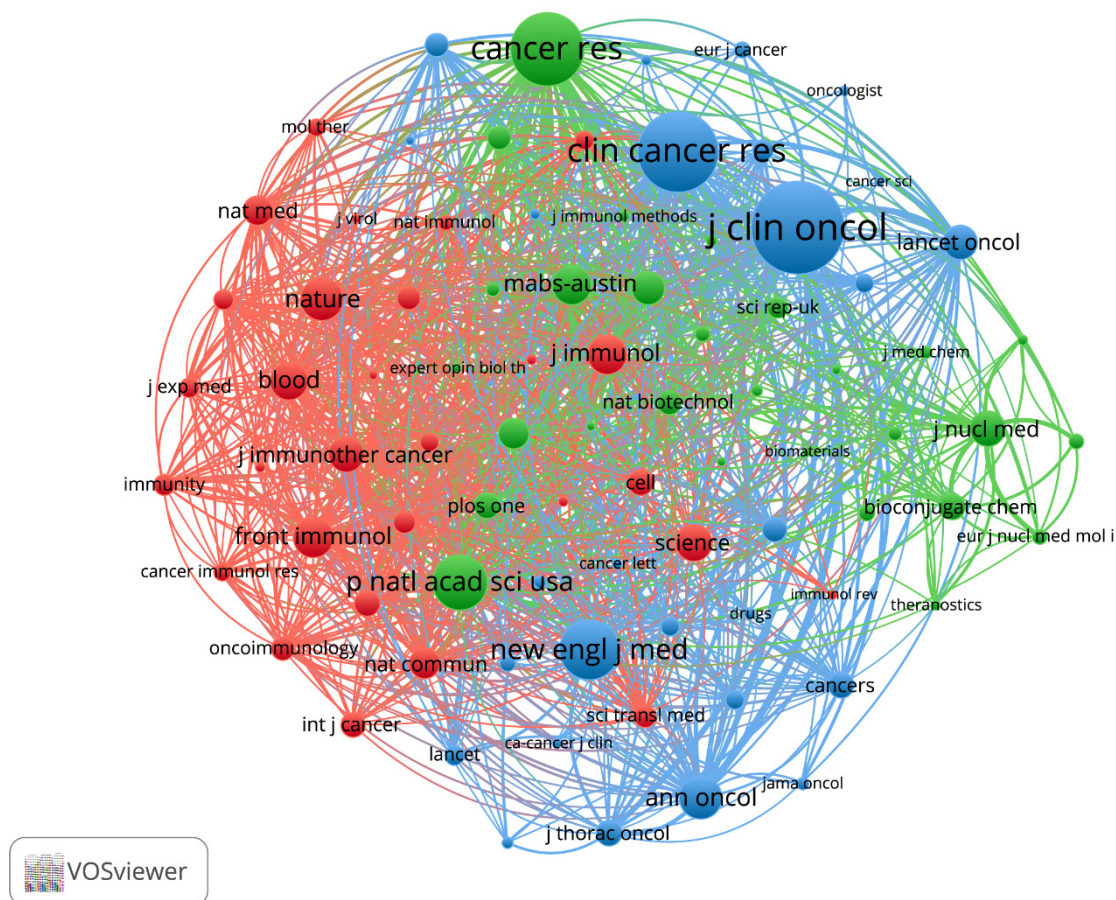


Figure 5B. Visualization of co-cited journals on bispecific antibody research in solid tumors.

The dual-map overlay of journals depicts the citation relationships and co-citation patterns, with citing journal clusters on the left and cited journal clusters on the right ^[21]. **Figure 6** illustrates three primary citation pathways. Research originating in molecular, biological, and genetic journals was primarily cited by work in

the molecular, biological, immunology, as well as medical and clinical fields. Similarly, articles published in health, nursing, and medical journals were predominantly cited by medical and clinical publications.

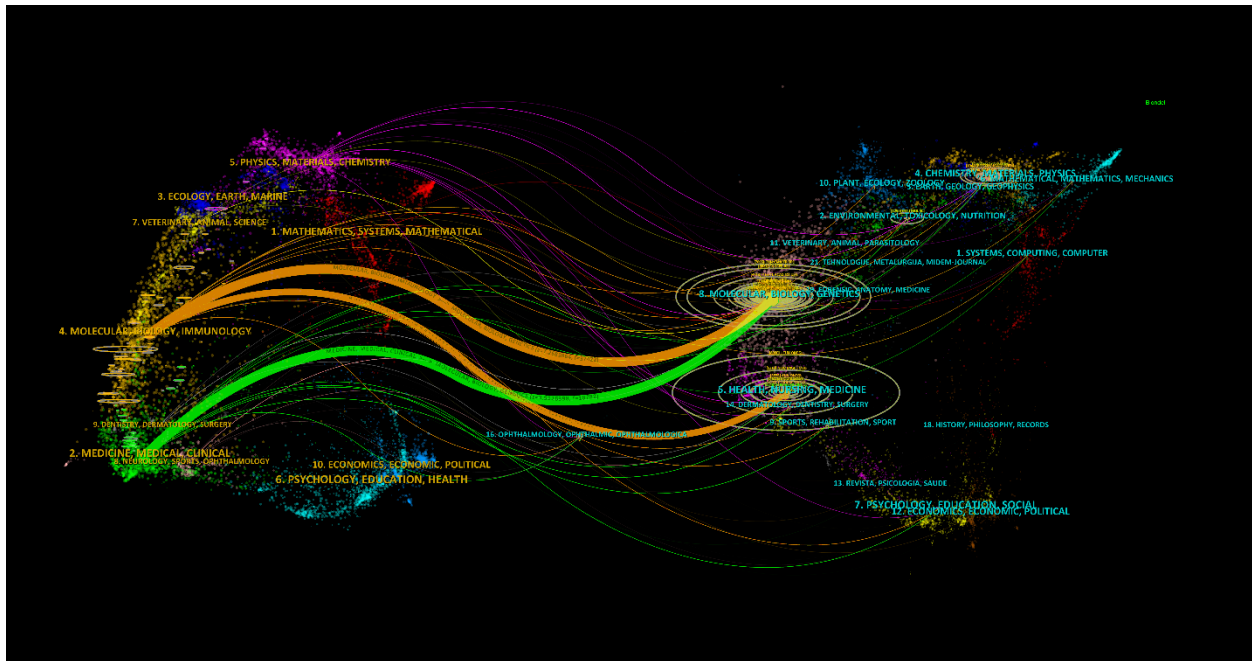


Figure 6. Dual-map overlay of journals on bispecific antibody research in solid tumors.

3.4. Authors and co-cited authors

A total of 20928 authors contributed to research on BsAbs in solid tumors. The top 10 most prolific authors each published over 20 relevant articles, with the top five being Christian Klein, Ryutaro Asano, Izumi Kumagai, Roland E. Kontermann, and Byoung Chul Cho (**Table 3**). A co-authorship network was constructed based on authors with a minimum of eight publications (**Figure 7A**). In the field of BsAb therapy for solid tumors, several distinct collaborative clusters have been formed around the aforementioned five core authors. Within these clusters, robust collaborative relationships exist among scholars, while collaborative ties of varying intensities are also present beyond these subnetworks. The cluster centered around Jessica C Hassel exhibits limited collaboration with other clusters, with its research primarily focusing on tebentafusp for uveal melanoma.

Among the 79691 co-cited authors identified, six were co-cited more than 250 times (**Table 3**). Aran F. Labrijn was the most frequently co-cited author (438 times), followed by Roland E. Kontermann (380 times) and Ulrich Brinkmann (272 times). Authors with at least 142 co-citations were included in the co-citation network analysis (**Figure 7B**). The co-citation network showed four closely related clusters: a red cluster led by Aran F. Labrijn and Roland E. Kontermann; a blue cluster centered on Pinky Sharma; a yellow cluster represented by Jing Li; and a green cluster with the core being Byoung Chul Cho.

Table 3. Top 10 authors and co-cited authors on bispecific antibody research in solid tumors

Authors	Publications	Co-cited Authors	Citations
Christian Klein	45	Aran F Labrijn	438
Ryutaro Asano	35	Roland E Kontermann	380
Izumi Kumagai	34	Ulrich Brinkmann	272
Roland E Kontermann	31	Byoung Chul Cho	268
Byoung Chul Cho	28	Patrick A Baeuerle	259
Pablo Umana	26	Jing Li	255
Wei Li	23	Luis Paz-Ares	246
Mitsuo Umetsu	22	Ming Yi	233
Jing Zhang	22	Pinky Sharma	217
Horst Lindhofer	22	Christian Klein	212

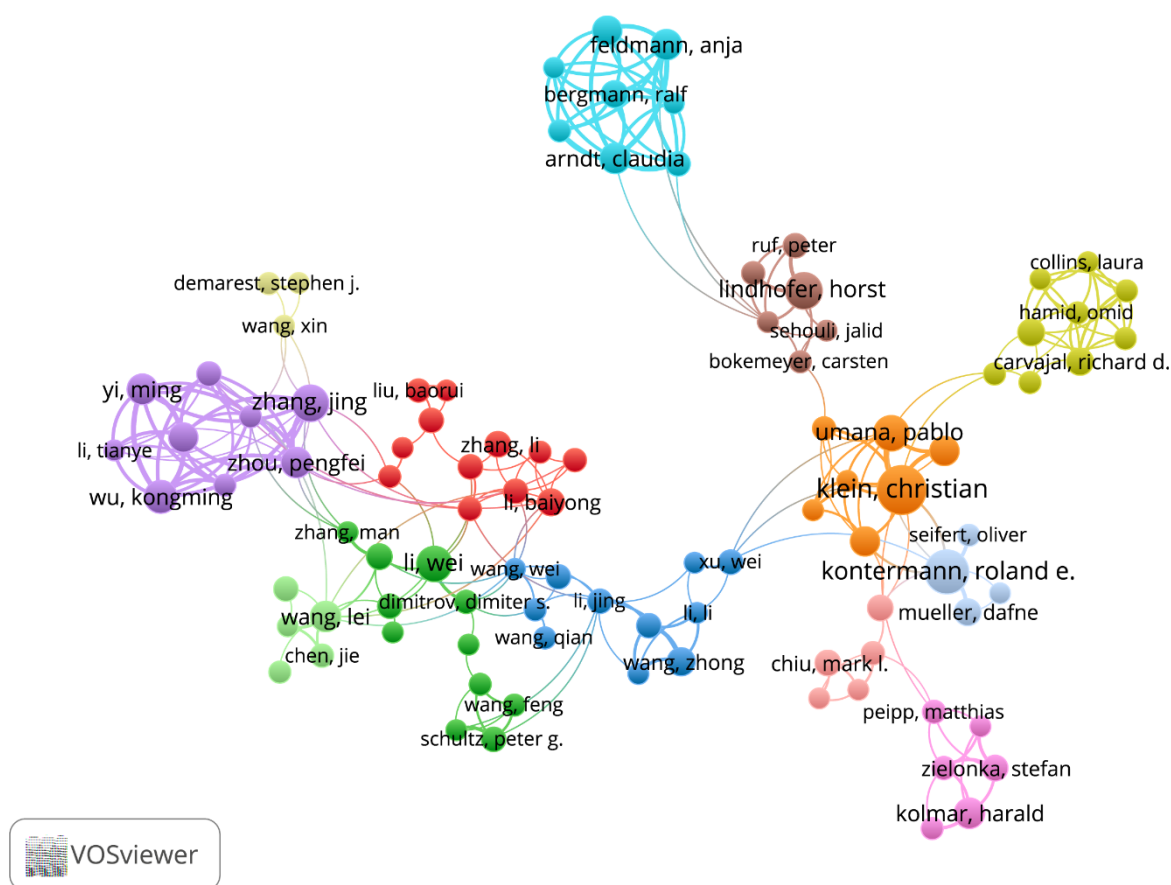


Figure 7A. Visualization of authors on bispecific antibody research in solid tumors.

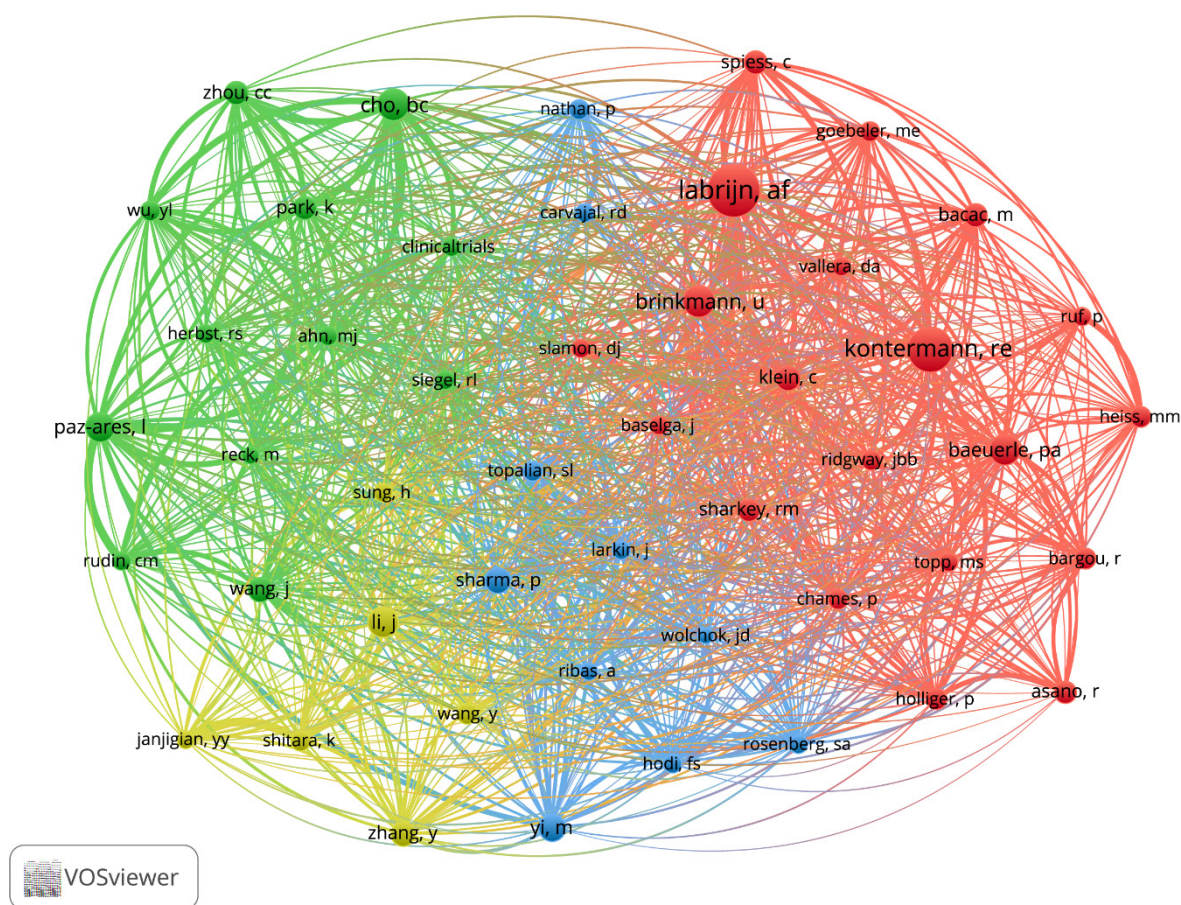


Figure 7B. Visualization of co-cited authors on bispecific antibody research in solid tumors.

3.5. Co-cited references

The analysis included the 132919 co-cited references during the last two decades of studies related to BsAbs for solid tumors. The top 10 references were co-cited 124 to 261 times (**Table 4**). The co-citation threshold was set at 77 to generate the co-citation network map (**Figure 8**), identifying several landmark publications serving to build basic knowledge of BsAbs. Articles by John B. B. Ridgway (1996), Ulrich Brinkmann (2017), and Aran F Labrijn (2019) demonstrated the highest co-citation frequencies, reflecting these authors' significant contributions to the structural engineering and clinical translation of BsAbs. Four major thematic clusters were identified through reference cluster analysis, including “Foundational research in antibody technology and biopharmaceuticals”, “Clinical translation of BsAbs”, “Catumaxomab”, and “Amivantamab”. The clear group structure and high overlap between fields suggest that molecular engineering, immunotherapy and targeted therapy are tightly integrated.

Table 4. Top 10 co-cited references on bispecific research in solid tumors

Co-cited references	Citations
Labrijn AF, 2019, Nat Rev Drug Discov, v18, p585 ^[22]	261
Brinkmann U, 2017, Mabs-Austin, v9, p182 ^[12]	224
Ridgway JBB, 1996, Protein Eng, v9, p617 ^[23]	169
Sung H, 2021, Ca-Cancer J Clin, v71, p209 ^[24]	154

Co-cited references	Citations
Nathan P, 2021, New Engl J Med, v385, p1196 ^[25]	144
Park K, 2021, J Clin Oncol, v39, p3391 ^[26]	143
Baeuerle PA, 2009, Cancer Res, v69, p4941 ^[1]	142
Spiess C, 2015, Mol Immunol, v67, p95 ^[7]	134
Kontermann RE, 2015, Drug Discov Today, v20, p838 ^[27]	131
Merchant AM, 1998, Nat Biotechnol, v16, p677 ^[28]	124

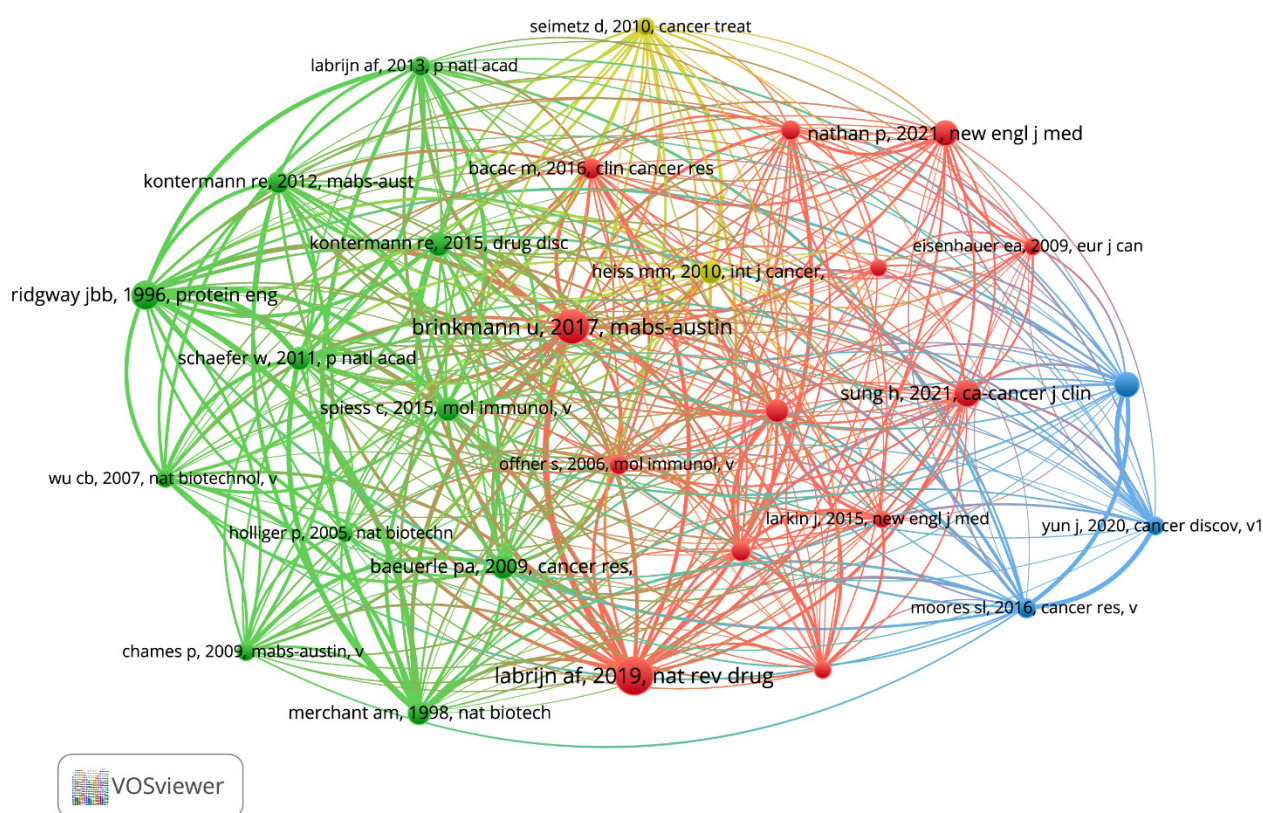


Figure 8. Visualization of co-cited references on bispecific antibody research in solid tumors.

3.6. References with citation bursts

The identification of the 25 references with the strongest citation bursts (**Figure 9**) highlights a selection of the most influential publications that achieved rapid and significant academic influence. The strongest citation bursts were associated with articles by Aran F Labrijn (2019, *Nat Rev Drug Discov*), Ulrich Brinkmann (2017, *MABs*), and Christoph Spiess (2015, *Mol Immunol*). These three publications were all reviews that systematically summarized the research and development of BsAbs, with emphasis on structural design, production and clinical applications, respectively. More recently, studies by Paul Nathan (2021), Keunchil Park (2021), and Hyuna Sung (2021) have demonstrated vigorous citation bursts, underscoring the growing focus on pivotal clinical trials that led to the regulatory approval of BsAbs for solid tumors. These trials included tebentafusp for metastatic uveal melanoma and amivantamab for EGFR exon 20 insertion-

mutated non-small cell lung cancer (NSCLC).

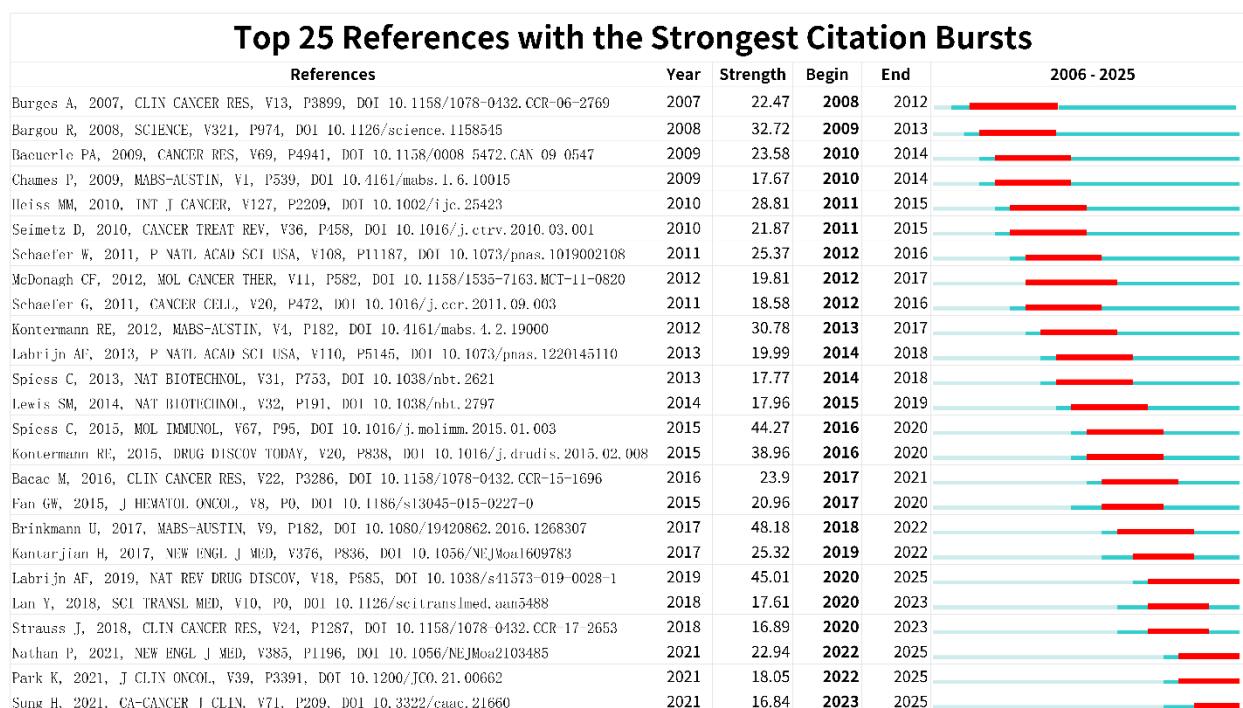


Figure 9. Top 25 references with the strongest citation bursts between 2006 and 2025.

3.7. Research hotspots and emerging trends

After the consolidation of synonymous terms, the 20 most frequently co-occurring keywords were obtained (Table 5). Major keywords included “BsAb” (n = 616), “immunotherapy” (n = 549) and “cancer” (n = 150), consistent with key topics of this field. It can be observed that “non-small cell lung cancer” stood out among keywords in terms of solid tumor types, suggesting an accelerated pace of BsAb research in this highly prevalent disease.

Table 5. Top 20 keywords on bispecific antibody research in solid tumors

Rank	Keywords	Counts	Rank	Keywords	Counts
1	Bispecific antibody	616	11	PD-L1	80
2	Immunotherapy	549	12	tumor microenvironment	78
3	Cancer	150	13	amivantamab	70
4	Cancer immunotherapy	143	14	immune checkpoint inhibitors	70
5	Non-small cell lung Cancer	117	15	bispecific	69
6	Targeted therapy	106	16	PD-1	63
7	Antibody	103	17	lung cancer	54
8	EGFR	103	18	antibody engineering	52
9	HER2	96	19	cd3	52
10	Breast cancer	87	20	monoclonal antibody	52

The analysis of keywords meeting the minimum occurrence threshold of 29 identified six main clusters (Figure 10A): The green cluster focuses on immunotherapy and tumor microenvironment (e.g., PD-1/

PD-L1 pathway), the red cluster centers on BsAbs (e.g., HER2/CD3-targeted agents), the blue cluster targets lung cancer therapy (specifically an anti-EGFR/c-MET [cellular-mesenchymal epithelial transition factor] BsAb for NSCLC), the purple cluster involves site-specific cancers (colorectal and prostate cancer), radioimmunotherapy with retargeting strategies, the cyan cluster centers on tebentafusp-mediated treatment for uveal melanoma, and the yellow cluster covers general targeted therapy and clinical trials. The hot spots represented by the core nodes (e.g., BsAb and immunotherapy) describe the major research themes for this field. Dense internal links and close nodes within clusters reflect the depth of studies on one theme, while cross-cluster links indicate synergistic integration between themes, which depicts a mature research ecosystem on target identification, drug development, and clinical translation.

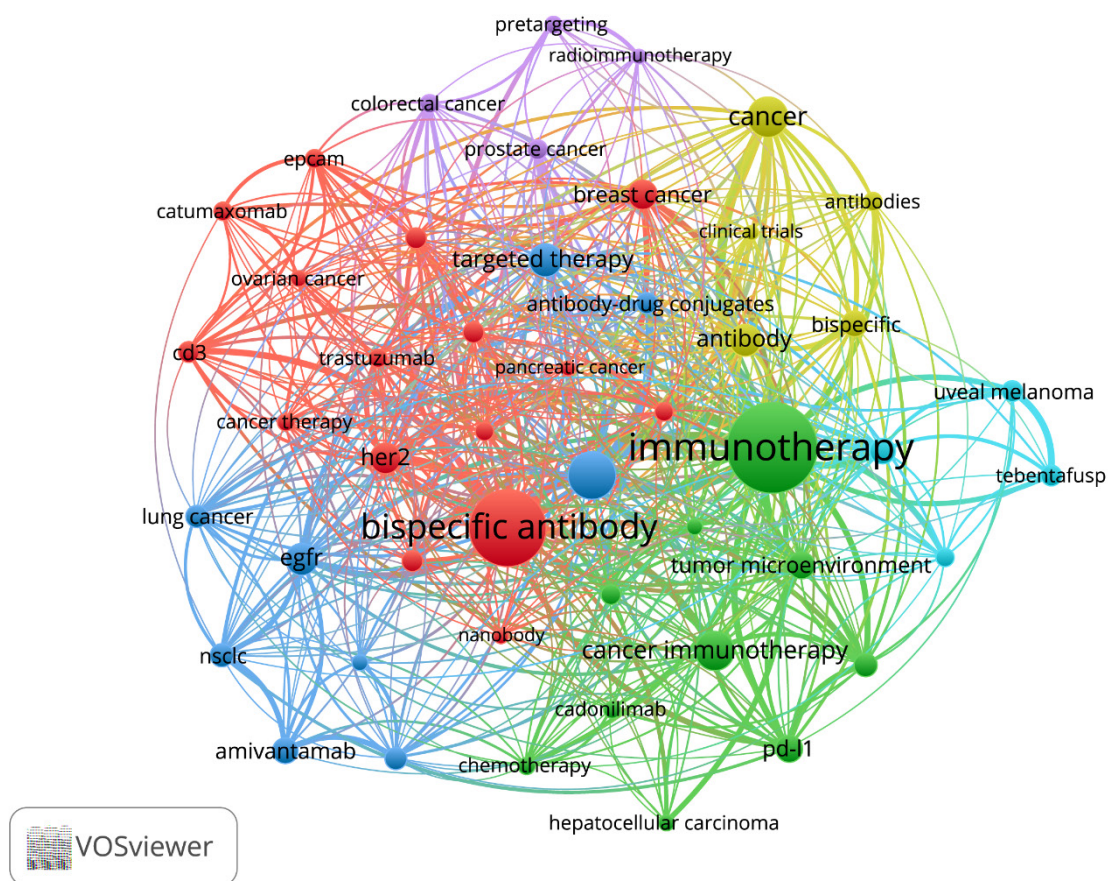


Figure 10A. Visualization of keywords with co-occurrence frequencies ≥ 29 .

The top 25 keywords that exhibited the strongest citation bursts are shown in Figure 10B. A citation burst analysis revealed the emergence of keywords with a marked surge in citation frequency, as well as the duration of the citation bursts. The early bursts (2006–2010) identified the emergence of core antibody technology (monoclonal-antibody, strength = 27.17) and preclinical model (tumor-cells, ovarian cancer). The mid-stage (2011–2020) transitioned towards BsAb design and production technology (antibody engineering) and a specific BsAb (catumaxomab). The recent (2022–2025) bursts (open label, uveal melanoma) evidenced their clinical translation (trial design and clinical practice). Overall, it indicates the transition from basic

research to clinical translation, which steers BsAb therapy in solid tumors. The temporal evolution of keywords, as analyzed by trend topic analysis (**Figure 10C**), demonstrates a sustained upward trend in the frequency of BsAb-related terms from 2008 to 2024. This growth is matched with concurrent advances in immunotherapy and targeted therapy. The emergence of drug-specific topics (e.g., amivantamab and cadonilimab) reflects accelerated clinical translation. Overall, this trend indicates increasingly intensive BsAb research in solid tumors and rapid progression toward clinical application.

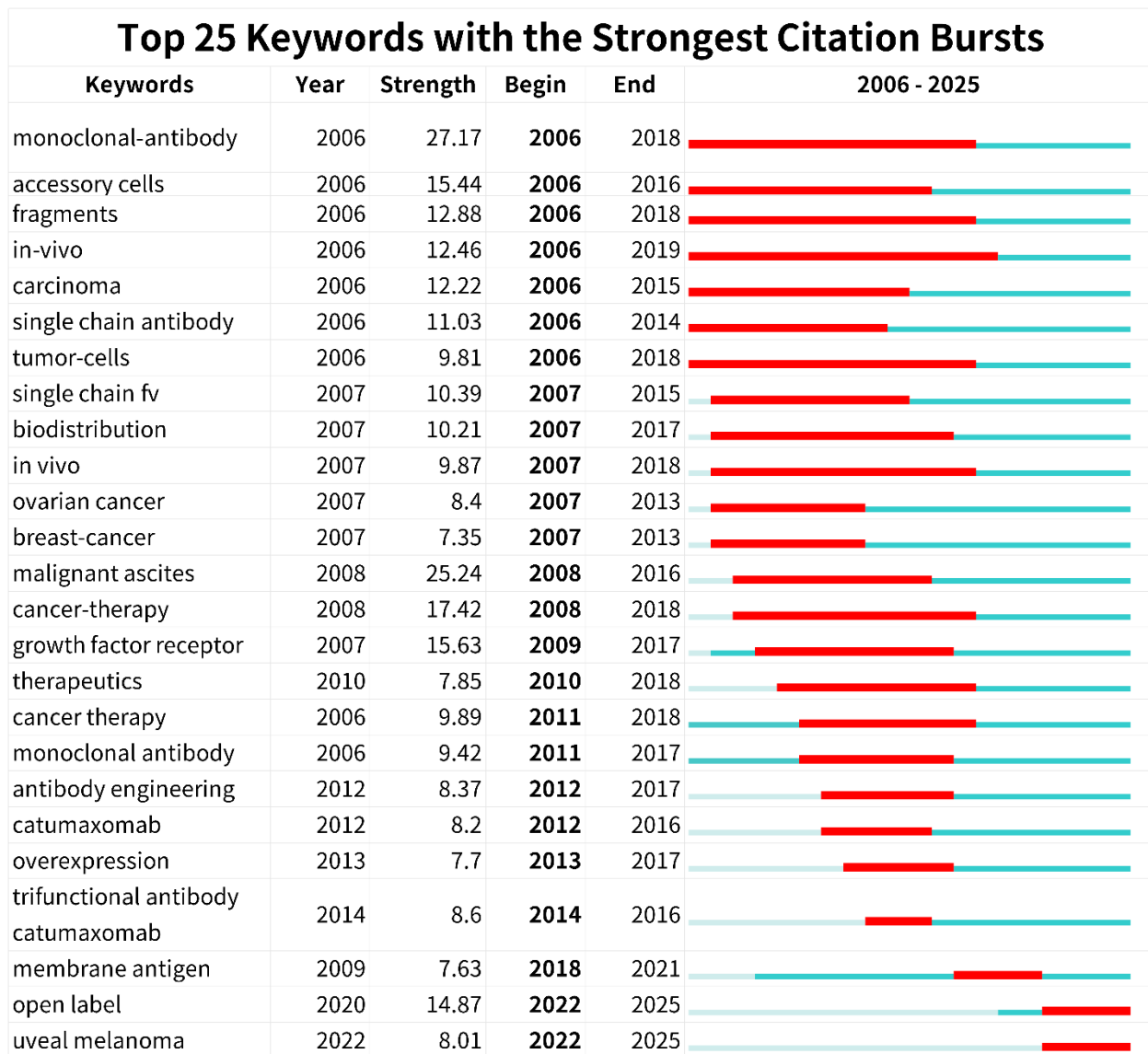


Figure 10B. Top 25 keywords with the strongest citation bursts between 2006 and 2025.

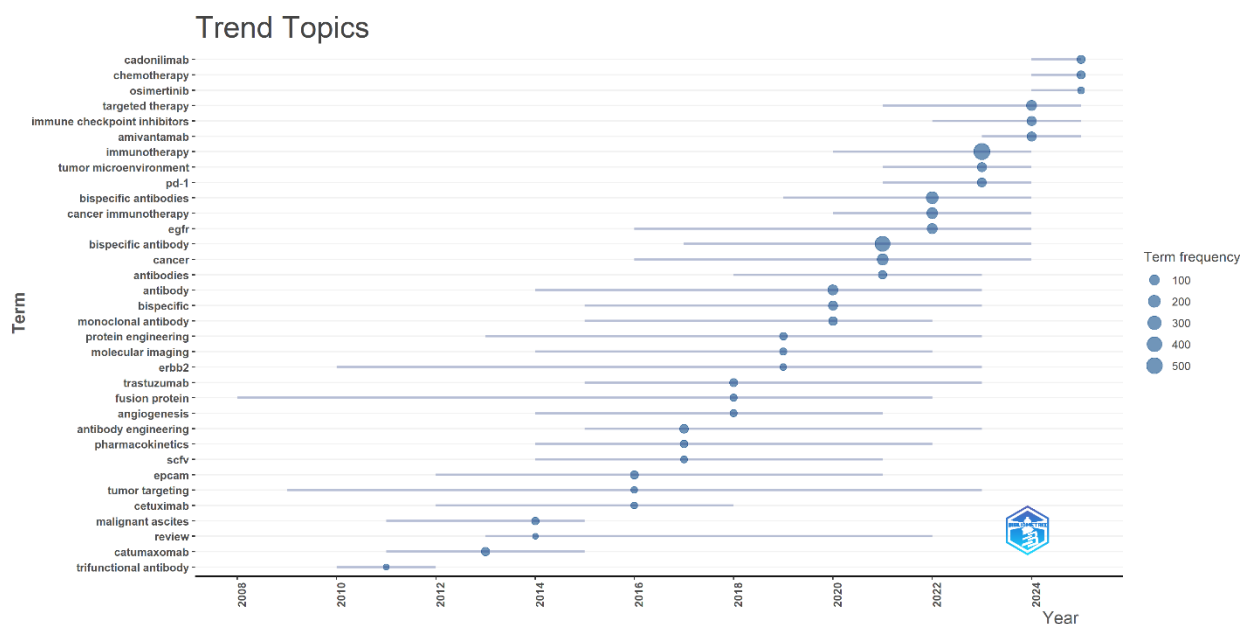


Figure 10C. Trend topic analysis on bispecific antibody research in solid tumors.

4. Discussion

BsAbs entered clinical trials in the 1990s^[29]. With advances in genetic engineering technologies, the stability and immunogenicity of BsAbs have been substantially optimized. In 2014, blinatumomab received regulatory approval and marked the beginning of the bispecific T-cell engager (BiTE) era^[15], laying the foundation for the rapid development of subsequent BsAbs. Compared with the huge success in hematologic malignancies, the use of BsAbs in solid tumors has developed relatively slowly. While catumaxomab was the first BsAb approved globally (for intraperitoneal therapy of malignant ascites), it was withdrawn for commercial reasons in 2017. Multiple approvals of BsAbs for solid tumors have only been seen in recent years. Four BsAbs were approved in 2024 for small cell lung cancer, NSCLC, biliary tract cancer and pancreatic cancer, respectively^[2,17–19]. Currently, BsAbs have entered a phase of broad approval in China and the US, with numerous promising candidates advancing through phase III randomized controlled trials (RCTs)^[10]. Therefore, the present bibliometric analysis was undertaken to provide a comprehensive overview of the research landscape, including its scale, pace of development, evolutionary trends and frontiers.

This study was conducted based on 3632 articles. The trend of yearly publications (**Figure 2**) showed that there was a significant rise in the number of relevant articles, with almost 60 % of total publications made in the last five years (2021–2025). This not only indicates the general scientific development trend, but is closely related to scientific breakthroughs of key technologies and clinical translation. A polynomial growth model ($R^2 = 0.9712$) strongly suggests that this expansion is systematic and sustained, driven by breakthroughs in antibody engineering, including robust platforms to ensure heavy-light chain pairing^[30] and, as of recently, by the groundbreaking clinical approvals such as tebentafusp^[25] and amivantamab^[26]. This trend suggests that the field has reached an era of robust clinical trial validation and drug discovery from the experimental proof-of-concept phase.

Our analysis reveals a global research landscape characterized by pronounced geographic concentration and distinct national roles. The US and China together contributed nearly two-thirds of the publication

output. This could be due to several factors: substantial and sustained public and private funding for biomedical research, large patient populations allowing quick progress of clinical trials, and national policies that prioritize biopharmaceutical innovation ^[27,31]. In the global collaboration network (**Figure 3**), the US clearly acts as a core of the global biopharmaceutical industry with huge collaborations with other countries. In contrast, despite China's rise as an influential contributor in BsAb studies, its international connections seem to be weaker. A possible explanation is its large and self-sufficient domestic research environment ^[32], language and policy barriers, and the more recent integration of China's pharmaceutical innovation system into the global network ^[33].

At the institutional level, Memorial Sloan Kettering Cancer Center, National Cancer Institute, and Sun Yat-Sen University demonstrated the highest impact. The leadership of these academic medical centers indicates that the advancement of BsAbs in solid tumors is fundamentally a translational process dependent on the close integration of basic science, preclinical models, and early phase clinical trials ^[34]. The observed collaboration between academic institutions and pharmaceutical companies indicates a symbiotic model in which academic institutions drive target discovery and mechanistic understanding, while the industry provides the engineering scale, manufacturing expertise, and development capital required to commercialize complex biologic drugs. This type of academic-clinical-industrial triangle seems to be an important source of innovation in this domain ^[35].

Journal and co-citation analyses further elucidate the field's knowledge dissemination and validation pathways. The high productivity of journals such as *Front Immunol* and *MAbs* reflects the field's strong roots in immunology and antibody engineering ^[3]. In contrast, the most influential co-cited journals, such as *J Clin Oncol* and *N Engl J Med*, are high-impact platforms for clinical trial result reporting. This contrast illustrates a clear knowledge flow: foundational research published in specialized journals ultimately seeks validation in high-impact clinical publications. The co-citation clusters confirmed that fundamental mechanisms ("how BsAbs work"), clinical efficacy ("if they work in patients"), and specific drug applications ("where they work") are closely interconnected.

Our results on the co-authorship and the co-citation networks give complementary insights into the intellectual and collaboration architecture of the area. The co-authorship network revealed a fragmented landscape composed of tightly knit clusters with limited bridging between them, as seen in the relatively isolated cluster focused on tebentafusp. This indicates that, although research and cooperation seem to be strong in subfields, they could benefit from more collaboration across these subfields. On the contrary, we note a tightly interconnected knowledge basis from the co-citation network. Authors like Aran F. Labrijn and Roland E. Kontermann play the most central intellectual roles, and researchers in different subfields share the same basis of theory and methodology. The field's development process is grounded in a unified body of knowledge, and we speculate that it could be accelerated by strategic collaborations between subfields. Fostering such partnerships between distinct yet intellectually proximate groups could be the key to catalyzing future breakthroughs.

Co-cited references are publications cited together in other papers. An analysis of these references can demonstrate the connections between publications and help establish a field's knowledge base ^[36]. This analysis is therefore crucial for researchers to identify key achievements and evaluate the impact of specific studies in their fields. The top 10 co-cited references were predominantly landmark reviews or key clinical trials in the field of BsAbs. The most co-cited publication was the review by Labrijn et al. (2019) ^[22], which

provided a mechanistic classification of BsAbs and a summary of BsAbs' development process and current state, established an explicit classification and assessment framework. The review by Brinkmann et al. (2017) ^[12] categorized, analyzed, and comparatively discussed a variety of antibody formats in a systematic manner to give the theoretical basis and roadmap for research on BsAbs. Both works have made tremendous contributions to translating BsAbs from bench to bed. Ridgway et al. employed an “knobs-into-holes” (KiH) approach that achieved efficient and specific heterodimerization of antibody heavy chains, thereby laying a solid technological foundation for the subsequent development of BsAb platforms ^[28]. Merchant et al. combined KiH technology with an engineered disulfide bond and solved the issue of light-chain mispairing, resulting in a complete platform directly applicable to BsAb manufacturing. The combination of these two papers represents the technological foundations for current BsAb manufacturing. Tebentafusp, a first-in-class BiTE able to redirect T cells to gp100-expressing melanoma cells, has led to a strong overall survival benefit for uveal melanoma patients in a phase III RCT ^[25]. Amivantamab, a BsAb that simultaneously binds to EGFR and c-MET, demonstrated strong efficacy against EGFR exon 20 insertion mutations in the Chrysalis trial ^[4], resulting in its accelerated approval by the US Food and Drug Administration (FDA) for patients progressing on platinum-based chemotherapy. This established it as the first targeted therapy approved for this population in the US. Subsequent trials evaluating its use in the first-line setting for both EGFR exon 20 insertion mutations ^[37] and EGFR sensitizing mutations ^[38] have also reported positive outcomes, further supporting its clinical utility. Articles on the two clinical trials that led to the regulatory approval of tebentafusp and amivantamab were also ranked among the top 10 co-cited references. The growing involvement of researchers and the availability of established technology platforms are driving an increasing number of BsAbs in the clinical development of solid tumors. The presence of citation bursts in references is a key indicator of emerging research topics, as it reflects a sharp, recent increase in scholarly attention within a specific field ^[39]. As shown in **Figure 9**, the initial citation burst emerged from an article published in 2007 ^[40], which reported the phase I/II trial results of catumaxomab for malignant ascites in ovarian cancer and lasted until 2012. Citation bursts beginning around 2015 were mainly from review articles that discussed the design, formats, and early clinical investigations of BsAbs ^[8,41,42]. Meanwhile, the ongoing citation bursts associated with the literature on tebentafusp and amivantamab indicate their substantial academic and clinical impacts. Research frontiers shift from the design and production of BsAbs to their clinical use.

The keyword analysis provides important information on the focus and evolution of BsAb research in solid tumors. After removing general terms such as “BsAb” and “cancer,” the predominant keywords identified include immunotherapy, targeted therapy, NSCLC, breast cancer, EGFR, and HER2 (**Table 5**). Based on their mechanisms of action, BsAbs in cancer therapy can be classified into two categories: the bridging type and the antigen cross-linking type ^[10]. Bridging-type BsAbs typically bind to antigens on both tumor cells and immune effector cells (e.g., T cells or natural killer cells), thereby activating effector cells to recognize and eliminate cancer cells ^[43]. In contrast, antigen cross-linking bsAbs simultaneously bind two distinct antigens or two different epitopes on a single antigen. This mechanism enables the effective co-inhibition of two disease-associated signaling pathways, such as immune checkpoint pathways (e.g., PD-1/CTLA-4) ^[44] or receptor tyrosine kinase pathways (e.g., EGFR and HER2) ^[44,45]. This likely accounts for the emergence of immunotherapy, targeted therapy, EGFR and HER2 as key terms in this field.

The keyword clustering provides a strategic framework for comprehending the disease-specific focus of BsAb research (**Figure 10A**). The clusters centered on NSCLC and HER2/CD3-targeted agents illustrate

a targeted research paradigm driven by major solid tumors. This pattern shows that research on BsAbs is progressing not as a pan-tumor agent, but rather focused towards specific oncological indications with huge unmet medical needs. The NSCLC cluster clearly indicates the rapid development of BsAbs directed against established tumor driver genes such as EGFR and c-MET ^[26], while the strong presence of HER2-directed BsAbs underscores their promising prospects in breast and gastric cancers ^[45,46]. This knowledge would be valuable for researchers and clinicians as it illustrates that the clinical achievement of BsAbs relies inherently on the choice of validated tumor antigens ^[47].

The temporal evolution of research focus, as revealed by citation burst strength and trend topic analysis, provides a compelling narrative of the field's maturation. The early bursts in "tumor-cells" and "in vivo" reflect the necessary preclinical groundwork, establishing the efficacy and mechanism of action of BsAbs in vitro and in animal models ^[48]. The subsequent shift towards "antibody engineering" signifies a phase of process optimization, addressing early challenges such as product stability and manufacturability ^[49]. The most recent bursts, highlighting "open label" trials and specific cancers like "uveal melanoma," mark the field's arrival at the clinical translation stage.

Overall, the keyword analysis illustrates a research community that has successfully passed its foundational and engineering phases and is now deeply engaged in the complex process of clinical application. The main implications for future research include facilitating the engineering of novel BsAb formats for specific medical needs, prioritizing combination approaches to overcome immunosuppressive TME, and learning from successful clinical translation drugs (such as amivantamab and tebentafusp) to inform clinical trial designs.

This work offers a systematic bibliometric landscape of BsAb research in solid tumors, covering immunotherapy and targeted therapy, which may provide valuable information. Multiple bibliometric tools, including CiteSpace, VOSviewer, and the R package "bibliometrix," were utilized to enhance the accuracy and depth of our analysis. However, this study had several limitations. Firstly, the literature search was conducted within a single database (WoSCC). Despite its comprehensive coverage of high-impact journals, WoSCC may omit relevant articles indexed exclusively in other databases, such as PubMed and Scopus. Secondly, the inclusion of only English-language publications could introduce language bias, as it might not fully capture the global research landscape. Finally, the citation-based impact of high-quality studies published in recent years is likely underestimated because of the citation delay effect.

5. Conclusion

The present bibliometric study outlined the dynamic development pattern of BsAb research in solid tumors during the last 20 years, indicating that this field has been garnering growing interest worldwide, especially in the US and China. This reference analysis reveals that BsAbs have successfully advanced from fundamental research to clinical application in the setting of solid tumors. However, the number of approved drugs remains limited. To accelerate future progress, it is imperative to enhance international collaboration and foster synergistic partnerships across distinct research subfields. The keyword evolution analysis demonstrates a clear trend from antibody engineering and preclinical research to the current focus on certain clinical applications, particularly in malignancies such as NSCLC and HER2-positive cancers. This shift highlights that the clinical success of BsAbs is intrinsically linked to targeting validated tumor-specific

antigens within well-defined patient populations. Future work should involve more rigorous clinical trials to expand the indications of BsAbs in solid tumors and to investigate their mechanisms of resistance and combination therapy strategies.

Disclosure statement

The authors declare no conflict of interest.

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Analysis of Clinicopathological Features of Dual-Phenotype Hepatocellular Carcinoma

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Abstract: *Objective:* To investigate the clinical manifestations, histopathological features, and differential diagnosis of dual-phenotype hepatocellular carcinoma. *Methods:* Histopathological observation and immunohistochemical study were performed on 3 cases of dual-phenotype hepatocellular carcinoma. *Results:* The tissues of 3 cases of dual-phenotype hepatocellular carcinoma showed irregular, unevenly sized nests and trabeculae. One case was dominated by fibrous stroma with indistinct sinusoids, and 2 cases had obvious sinusoids. The cells were large, polygonal, with strong cell adhesion, relatively clear cell boundaries, and abundant and strongly eosinophilic cytoplasm. The cell nuclei exhibited significant pleomorphism and atypia, with thick nuclear membranes, coarse chromatin in clumps, obvious nucleoli, and visible mitoses. *Conclusion:* Dual-phenotype hepatocellular carcinoma is a unique and highly aggressive subtype of primary liver cancer, which is relatively rare and often occurs in patients with hepatitis and cirrhosis, with abdominal pain as the main clinical symptom. It has the biological behaviors of both hepatocellular carcinoma (HCC) and cholangiocarcinoma, and the clinical prognosis is poor.

Keywords: Dual-phenotype hepatocellular carcinoma; Histopathology; Diagnosis

Online publication: May 31, 2026

1. Introduction

Dual-phenotype hepatocellular carcinoma (DPHCC) was first reported in 2011 ^[1] and represents a unique and highly aggressive subtype of primary liver cancer. It has been clearly defined as an independent pathological type rather than a simple mixture of hepatocellular carcinoma (HCC) and cholangiocellular carcinoma. The incidence of DPHCC accounts for approximately 10.1% of HCC cases ^[2,3]. DPHCC can be diagnosed when the tumor exhibits a single histological component of HCC while strongly expressing ≥ 1 HCC marker and at least one cholangiocarcinoma marker in $>15\%$ of cancer cells ^[4]. Although DPHCC shares some histopathological similarities with typical HCC, it also exhibits distinct features and displays biological behaviors characteristic of both HCC and cholangiocellular carcinoma, leading to a poor clinical prognosis. The “Standardized Pathological Diagnosis Guidelines for Primary Liver Cancer (2015 Edition)” has incorporated DPHCC into routine pathological diagnosis ^[5]. This study retrospectively analyzed three

cases of DPHCC diagnosed at our hospital to explore the key points of diagnosis and differential diagnosis, immunophenotype, relevant treatment methods, and prognostic characteristics.

2. Objects and methods

2.1. Case selection

Three cases of DPHCC were selected from external examination cases in the Department of Tumor Pathology at Harbin Medical University Cancer Hospital between 2018 and 2025. There were two males and one female, with a male-to-female ratio of 2:1. The patients' ages ranged from 37 to 62 years old, with an average age of 50 years old.

2.2. Methods

Histological observation and immunohistochemical analysis were performed on the three DPHCC cases. Specimens were fixed in 10% neutral formalin, routinely dehydrated, embedded in paraffin, sectioned at 4 μ m thickness, and stained with HE for slide preparation and immunohistochemical staining. Routine HE staining and light microscopy were used for observation. Immunohistochemical staining was performed using the EnVision method with DAB chromogen and hematoxylin counterstaining. The antibodies used, including CK7, CK19, CK8/18, AFP, PAX-8, Arginase-1, HSP70, CD34, and Glypican-3 kits, were all purchased from Fuzhou Maixin Biotechnology Co., Ltd. CK19, CK7, AFP, CK8/18, and CK20 were expressed in the cytoplasm; Arginase-1 was expressed in the nucleus and cytoplasm; Glypican-3 was expressed in the cytoplasm and cell membrane; Hepatocyte, HSP70, and CD34 were expressed in the cytoplasm; Ki67 was expressed in the nucleus. Positive expression was indicated by brownish-yellow staining in the corresponding areas, with negative and positive controls established simultaneously.

3. Results

3.1. Clinical manifestations

The clinical manifestations of the cases in this group are as follows:

- (1) Case 1: The patient presented with intermittent right upper abdominal pain of no obvious cause 15 days prior to admission. An enhanced MR scan at our hospital revealed a space-occupying lesion in liver segments S4/S5, suggesting liver cancer with portal vein tumor thrombus. The patient was admitted with a diagnosis of "liver space-occupying lesion" and was in generally good condition. During surgery, no ascites was observed in the abdominal cavity, and no implant nodules were found on the peritoneum, pelvis, or greater omentum. The liver surface exhibited mixed-type cirrhosis with both large and small nodules, was dark red in color, and had a hard texture. Lesions were palpable in the left medial and right anterior lower segments of the liver, which were hard and consisted of scattered granular multiple tumors fused into a mass measuring approximately 9×7 cm. A tumor thrombus was palpable in the left hepatic duct. The spleen was enlarged, firm, and measured approximately 10×8×6 cm. A liver segment II, III, IV, and V resection was performed.
- (2) Case 2: Outpatient case with incomplete clinical data.
- (3) Case 3: The patient underwent a color ultrasound examination at an external hospital 6 days prior, which revealed multiple liver nodules, diffuse cirrhosis, cholecystitis, multiple gallstones, and splenomegaly.

An enhanced CT scan showed a nodule in liver segment S8, suggesting nodular carcinogenesis and splenomegaly with nodular cirrhosis. During surgery, no ascites was observed in the abdominal cavity, and no implant nodules were found on the peritoneum. The liver surface exhibited mixed-type cirrhosis with both large and small nodules, was dark red in color, and had a hard texture. Imaging revealed a tumor measuring approximately 16 mm in the parenchyma of liver segment S8. The gallbladder contained multiple stones. The spleen was significantly enlarged, and a total splenectomy, cholecystectomy, and ultrasound-guided radiofrequency ablation of the liver tumor were performed.

3.2. Gross examination of specimens

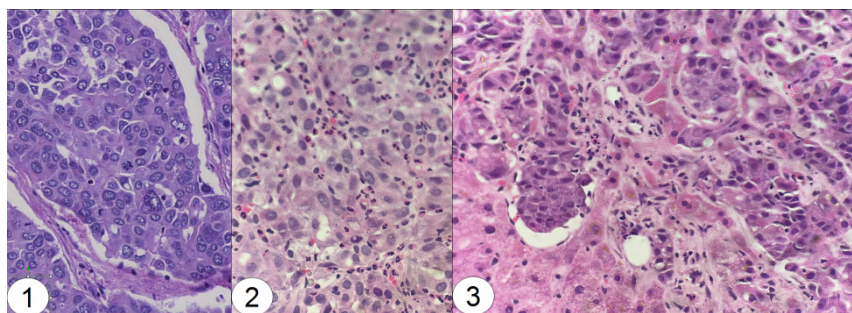
- (1) Case 1: The resected specimens included liver segments II, III, IV, and V, along with the gallbladder. The liver measured 103 mm × 60 mm × 50 mm, and a grayish-white rough area measuring 60 mm × 40 mm was visible on the liver capsule. Upon incision, a tumor measuring 60 mm × 60 mm × 30 mm was observed beneath the rough area, which was adherent to the gallbladder.
- (2) Case 2: The submitted specimen was a cord-like tissue with a total volume of approximately 2 mm × 2 mm × 1 mm and was grayish-white in color.
- (3) Case 3: The submitted specimen was a cord-like tissue with a total volume of approximately 7 mm × 1 mm × 1 cm and was grayish-white in color.

3.3. Microscopic findings

The cancerous tissue appeared as irregular, variably sized nests and clusters, predominantly composed of sinusoidal stroma. Some cancerous tissues exhibited obvious fibrous stroma, indicating the morphological diversity of DPHCC. The cells were large and polygonal, with strong cell adhesion, clear cell boundaries, abundant cytoplasm, and intense eosinophilia. The nuclei showed significant pleomorphism and atypia, with thick nuclear membranes, coarse chromatin, clumped appearance, distinct nucleoli, and visible mitotic figures. Infiltrative growth was observed at the edge of the tumor tissue into the surrounding normal tissue.

3.4. Immunohistochemistry

All three cases of DPHCC showed diffuse expression of CK7 and CK19. One case expressed AFP, two cases expressed Hepatocyte, and one case expressed HSP70. Arginase-1 was not expressed.



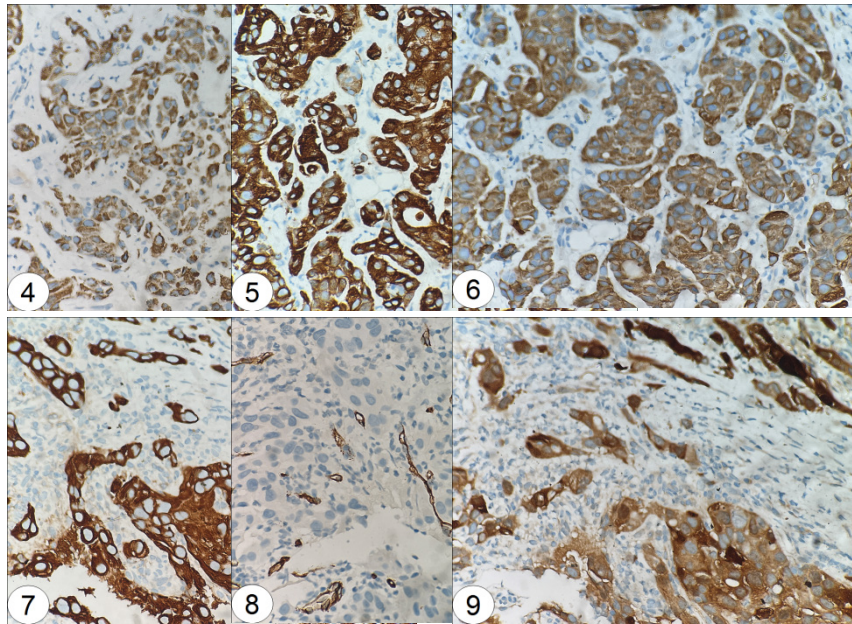


Figure 1. Tumor cells are arranged in nests and clusters with sparse interstitial fibers; **Figure 2.** Diffuse, ill-defined nests and clusters; **Figure 3.** Tumor tissue exhibits infiltrative growth into surrounding normal liver tissue; **Figure 4.** Diffuse cytoplasmic expression of Hepatocyte; **Figure 5.** Diffuse cytoplasmic expression of CK7; **Figure 6.** Diffuse cytoplasmic expression of CK19; **Figure 7.** Diffuse cytoplasmic expression of CK8/18; **Figure 8.** Interstitial sinusoids are not prominent; **Figure 9.** Diffuse cytoplasmic expression of HSP70.

4. Discussion

4.1. Clinical characteristics and pathogenesis

In China, the high prevalence of hepatitis leads to a relatively high incidence of hepatocellular carcinoma (HCC), which is characterized by high malignancy, recurrence, and mortality rates. Liver cancer ranks fifth in incidence and second in mortality among the most common malignancies in China ^[6]. The postoperative recurrence rate is as high as 70%, with most recurrences occurring within two years after surgery ^[2]. Clinically, DPHCC presents similarly to ordinary HCC without specific symptoms, commonly including right upper abdominal discomfort, pain, weight loss, and fatigue. Serum alpha-fetoprotein (AFP) levels are typically significantly elevated in patients. Some patients may also exhibit elevated CA19-9 levels, suggesting the presence of cholangiocellular differentiation components. On CT or MRI, DPHCC often manifests as atypical liver lesions, lacking the typical “fast-in, fast-out” enhancement pattern of HCC. Instead, it may show inhomogeneous arterial enhancement and persistent enhancement in the portal or delayed phases, resembling cholangiocarcinoma. The vast majority of HCC cases occur based on viral hepatitis and cirrhosis, with a strong correlation with hepatitis B virus infection (especially in Asia). Similar to conventional HCC, DPHCC predominantly affects middle-aged and elderly males. Therefore, when histopathological classification is challenging, especially with small biopsy specimens, clinical data, including AFP and CA19-9 levels, play a crucial role in accurate diagnosis. The incidence of DPHCC is higher in males than in females ^[7], which aligns with our data. DPHCC is a novel subtype of HCC first reported in 2011, accounting for approximately 10.1% of all HCC cases ^[3,4], although some reports suggest an incidence of 1–5%. Based on the number of cases retrieved from our hospital, the incidence is closer to

the latter estimate. The World Health Organization classifies primary liver cancer into three categories: HCC, intrahepatic cholangiocarcinoma (ICC), and mixed hepatocellular-cholangiocarcinoma^[8]. DPHCC is a new subtype separated from within the HCC category. The pathogenesis of DPHCC remains unclear, with two main hypotheses proposed. The first hypothesis suggests that DPHCC originates from hepatic progenitor cells (HPCs), which possess bidirectional differentiation potential into hepatocytes and cholangiocytes and serve as liver reserve cells^[9]. This indicates their stem cell-like properties: Lee JS et al.^[10] pioneeringly defined liver cancer expressing cholangiocellular markers (such as CK19) and stem cell markers (such as EpCAM) as a “hepatic progenitor cell-derived” subtype in 2006, revealing the cellular origin basis of its malignant biological behavior. Since DPHCC tumor cells can simultaneously express HCC and cholangiocellular markers, this hypothesis suggests that DPHCC tumor cells may originate from HPCs with bidirectional differentiation potential. The second hypothesis proposes dedifferentiation of HCC. Previous studies have found that although CK19 can be expressed in some HCC cells, it is negative in HCC precancerous lesions. Cells expressing CK19 are mature hepatocytes that gradually dedifferentiate during malignant transformation rather than originating from HPCs^[11].

4.2. Histopathology and immunohistochemistry

DPHCC shares some histopathological similarities with typical HCC. Well-differentiated DPHCC cells may form glandular, pseudoglandular, or delicate trabecular patterns; moderately differentiated DPHCC may exhibit relatively thick trabeculae; and poorly differentiated DPHCC may present as solid nests or sheets with indistinct trabecular structures. Hepatic sinusoid-like vascular networks are visible around the tumor, with minimal fibrous interstitium.

Tumor cells are typically polygonal or cuboidal with eosinophilic or clear cytoplasm, well-defined borders, abundant cytoplasm, and strong eosinophilia. Nuclear atypia ranges from moderate to severe, with common mitotic figures. In glandular regions, cells may appear columnar, resembling cholangiocytes. The tumor interstitium may be rich in fibrous connective tissue, similar to the “desmoplastic” reaction seen in cholangiocarcinoma, which differs from ordinary HCC. Accurate diagnosis of DPHCC requires immunohistochemical detection. When a tumor has a single HCC cellular component and strong positivity for one or more hepatocyte markers is observed in more than 15% of tumor cells, along with strong positivity for one or more cholangiocellular markers, DPHCC can be diagnosed. HepPar-1 typically shows strong, diffuse cytoplasmic granular staining, representing one of the most specific hepatocyte markers. Arginase-1 exhibits nuclear and cytoplasmic positivity with high sensitivity and specificity. AFP may be positive but is usually focal. Cholangiocellular markers (at least two positives), with CK7 and CK19 being the most commonly used combination.

4.3. Treatment and prognosis

Literature universally indicates that dual-phenotype liver cancer represents a “bottleneck” in liver cancer treatment, showing resistance to existing therapeutic approaches. Therefore, accurate pathological diagnosis and classification are crucial for prognosis assessment and the exploration of new treatment strategies. Currently, the primary treatment for DPHCC patients is complete surgical resection, supplemented by immunotherapy, targeted therapy, and chemotherapy when necessary. The overall survival and recurrence-free survival of DPHCC patients are lower than those of ordinary HCC patients^[3]. According to studies by

Kim H et al. and several similar investigations, CK19 positivity (especially when co-expressed with CK7) is an independent risk factor for poor prognosis in liver cancer patients, closely associated with high tumor aggressiveness, vascular invasion, and chemotherapy resistance. Overall, the prognosis is extremely poor. Numerous studies confirm that the prognosis of dual-phenotype liver cancer is significantly worse than that of ordinary HCC and combined hepatocellular-cholangiocarcinoma. Its aggressive behavior is characterized by higher early recurrence rates, higher lymph node metastasis rates, and a greater tendency for distant metastasis. Patients' overall survival is significantly shortened.

5. Differential diagnosis

5.1. Combined hepatocellular-cholangiocarcinoma

In combined carcinomas, hepatocellular and cholangiocellular components are spatially separated and coexist, containing distinct histological components of HCC and cholangiocarcinoma, each expressing their respective immunomarkers^[12,13]. In contrast, dual-phenotype liver cancer consists of a single tumor cell population with dual hepatocellular and cholangiocellular phenotypes, reflecting tumor cell dedifferentiation and stem cell-like properties.

5.2. Classic hepatocellular

Carcinoma with CK19 Expression Approximately 10-30% of ordinary HCCs may focally express CK19 ("stem cell-related subtype"), but they typically do not express or only express CK7 in a minority of cells. Dual-phenotype liver cancer requires co-expression of CK7 and CK19.

5.3. Intrahepatic cholangiocarcinoma

Cholangiocarcinoma expresses CK7 and CK19 but does not express HepPar-1 and Arginase-1. Occasionally, weak positivity or false positivity for HepPar-1 may occur, but Arginase-1 exhibits extremely high specificity.

5.4. Metastatic hepatoid adenocarcinoma

Metastatic hepatoid adenocarcinoma is a rare, poorly differentiated adenocarcinoma with histological and immunohistochemical similarities to HCC. Although its histological and immunophenotypic features overlap with DPHCC, metastatic hepatoid adenocarcinoma originates from organs outside the liver and typically expresses SALL4, allowing differentiation from DPHCC^[14].

6. Conclusion

Dual-phenotype hepatocellular carcinoma is a unique and highly aggressive subtype of primary liver cancer. According to the 2019 WHO Classification of Tumours of the Digestive System, it is clearly defined as an independent pathological type rather than a simple mixture of HCC and cholangiocarcinoma. Its most significant feature is the simultaneous expression of HCC and cholangiocarcinoma immunophenotypes by the same tumor cell population. Compared to ordinary HCC, DPHCC exhibits higher malignancy and worse prognosis. It is associated with higher rates of microvascular invasion, intrahepatic and extrahepatic metastasis, and postoperative recurrence. The classification of DPHCC is of great significance for precise treatment and prognosis assessment in HCC patients. Due to the limited extent of current research on

DPHCC, misdiagnosis or missed diagnosis often occurs in clinical practice.

Disclosure statement

The authors declare no conflict of interest.

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Clinical Review on Perioperative Immunotherapy Patterns for Non-Small Cell Lung Cancer

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Abstract: The application scope of immunotherapy has gradually expanded from advanced non-small cell lung cancer to the perioperative stage, with three core clinical application forms progressively established: neoadjuvant therapy, adjuvant therapy, and comprehensive management combining neoadjuvant and adjuvant approaches. Recent clinical studies have demonstrated that all three treatment modalities can achieve improvements in pathological response and event-free survival. However, consensus remains elusive regarding the characteristics of beneficiary populations, efficacy differences among various treatment strategies, and perioperative-specific safety risks. By integrating recent clinical research findings and systematically reviewing evidence-based data for each therapeutic approach, this review aims to provide evidence-based support for rational clinical treatment selection.

Keywords: Non-small cell lung cancer; Perioperative period; Immunotherapy; Treatment modality; Immune-related adverse reactions

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1. Introduction

Lung cancer ranks as the most prevalent and lethal malignant tumor globally, with non-small cell lung cancer (NSCLC) accounting for over 80% of cases. Radical surgery remains the standard treatment for resectable cases, yet high recurrence rates persist postoperatively, and conventional perioperative chemotherapy demonstrates limited efficacy in improving long-term survival outcomes. The advent of immune checkpoint inhibitors has introduced novel therapeutic approaches, with multiple studies validating their significance in perioperative applications. Critical challenges in current clinical practice include rational selection among neoadjuvant therapy, adjuvant therapy, and neoadjuvant-consolidation regimens, precise identification of

high-risk populations, and effective management of perioperative-specific adverse reactions.

2. Evidence-based foundations of three models for perioperative immunotherapy

2.1. Research evidence on neoadjuvant immunotherapy regimens

Wang Changli (2022) conducted a systematic review of key studies in this field. As the first Phase III clinical trial, CheckMate816 demonstrated improved pathological complete response rates and event-free survival following neoadjuvant nivolumab combined with chemotherapy. Hao Zhuohong (2024) analyzed trials such as NADIM and NADIMII, revealing that patients in Phase IIIIA maintained high primary pathological response rates after receiving combined immunotherapy and chemotherapy regimens. Hong Wen yuan (2024) discussed NEOSTAR study data, indicating that the dual immunotherapy regimen combining nivolumab and ipilimumab holds promising potential for improving pathological responses. Xu Yuanyuan (2024) investigated early-stage studies on immunotherapy monotherapy, with CheckMate159 trial data confirming that neoadjuvant immunotherapy monotherapy achieves pathological response without affecting surgical procedures.

2.2. Research evidence on adjuvant immunotherapy modalities

Adjuvant immunotherapy can be utilized to eliminate minimal residual disease postoperatively and reduce the likelihood of tumor recurrence ^[1]. Wang Changli (2022) reviewed the results of two Phase III studies, IMpower010 and KEYNOTE-091, demonstrating that atezolizumab application in programmed death ligand 1-positive patient populations prolongs disease-free survival, while pembrolizumab exhibits positive effects on overall disease-free survival across the entire study cohort. Xu Yuanyuan (2024) analyzed the inclusion criteria and key observation indicators of both studies, noting that the adjuvant chemotherapy followed by a sequential immunotherapy regimen adopted in IMpower010 has become the current mainstream clinical approach ^[2]. Hong Wen yuan (2024) highlighted in his discussion that patients without prior adjuvant chemotherapy in the KEYNOTE-091 study showed no significant benefit from pembrolizumab treatment, thereby validating the clinical value of prior adjuvant chemotherapy.

2.3. Research evidence on the “sandwich cake” perioperative whole-process treatment model

The “sandwich model” represents a comprehensive treatment strategy integrating neoadjuvant immunotherapy, surgery, and adjuvant immunotherapy ^[3]. Fang Yujia (2024) published findings from the AEGEAN study, demonstrating that perioperative application of davolizumab can prolong event-free survival and improve pathological complete response rates. Hong Wen yuan (2024) analyzed data from multiple Phase III trials, including KEYNOTE-671, Neotorch, and RATIONALE-315, revealing that the pembrolizumab group exhibited superior outcomes compared to the placebo group in both event-free survival and primary pathological response rates ^[4]. Hao Zhuohong (2024) elucidated the event-free survival benefits observed in the netreplisumab group within the Neotorch study and identified correlation data between perioperative tislelizumab administration and primary pathological response rates in the RATIONALE-315 trial. Wang Chenming (2025) synthesized these research findings to establish core evidence for perioperative treatment, with this model consistently demonstrating therapeutic benefits across multiple clinical studies.

3. Screening of beneficiary populations for different treatment modalities

3.1. The predictive value and controversies of PD-L1 expression levels

The expression of programmed death ligand 1 (PD-L1) demonstrates robust predictive efficacy during the neoadjuvant therapy phase. Wang Changli (2022) reported in the CheckMate816 study that there is a strong correlation between patient treatment benefit and PD-L1 expression levels, with increased PD-L1 levels correlating with greater treatment efficacy. However, the predictive value of this biomarker in adjuvant therapy remains controversial. Liu Xuehui (2025) systematically reviewed these discrepancies, noting that IMpower010 data suggest higher treatment benefits in high-PD-L1 expression populations, whereas the KEYNOTE-091 study failed to demonstrate statistical significance in the endpoint metrics of the high-PD-L1 subgroup ^[5]. Xu Yuanyuan (2024) proposed that the KEYNOTE-091 study revealed a survival advantage shift in the control cohort, which may have directly obscured the statistical differences between groups ^[6]. The expert consensus led by Xie Qichao (2022) concluded that the reference value of PD-L1 expression levels for adjuvant immunotherapy benefits has not yet reached a unified consensus, underscoring the need for further clinical studies for validation.

3.2. Impact of clinical staging and pathological type on therapeutic efficacy

Wang Changli (2022) mentioned in the CheckMate816 study that patients in stage III achieved higher benefits after neoadjuvant immunotherapy compared to those in stage IB-II. Liu Xuehui (2025) analyzed subgroup data from the AEGEAN study, demonstrating improved pathological responses in both stage II and stage III patients following neoadjuvant immunotherapy, though no definitive conclusion was reached regarding the benefits across stages. At the pathological type level, existing studies have not reached a unified conclusion. Liu Xuehui (2025) reviewed multiple studies, revealing that the Neotorch study showed superior event-free survival benefits in squamous cell carcinoma patients compared to non-squamous cell carcinoma, while the CheckMate816 study demonstrated more pronounced benefits in non-squamous cell carcinoma. However, long-term follow-up data from this study showed no statistically significant difference in overall survival between squamous cell carcinoma and non-squamous cell carcinoma.

3.3. The potential of ctDNA-MRD as a novel biomarker

The clearance status of circulating tumor DNA (ctDNA) is closely associated with immunotherapy efficacy. Wang Changli (2022) observed in the CheckMate816 study that the clearance rate of ctDNA under neoadjuvant immunotherapy combined with chemotherapy was higher than that of chemotherapy alone, with patients achieving clearance demonstrating superior pathological complete response rates. Xu Yuanyuan (2024) validated through the PROSPECTIVE LUNGCA-1 study data that postoperative molecular residual disease positivity serves as a key prognostic indicator for recurrence risk, with positive groups exhibiting significantly higher recurrence rates compared to negative groups. Liu Xuehui (2025) proposed that cases maintaining negative postoperative ctDNA molecular residual disease demonstrate potential cure characteristics, where adjuvant therapy may yield limited additional benefits. Xie Qichao (2022) suggested in a relevant expert consensus that populations with detection capabilities could utilize dynamic molecular residual disease monitoring to guide treatment planning, emphasizing that even late-stage cases should receive adjuvant therapy despite negative molecular residual disease results.

4. Key issues in selection of perioperative immunotherapy regimens

4.1. Consideration of neoadjuvant therapy cycles and surgical timing

The optimal number of cycles for neoadjuvant immunotherapy remains inconclusive. Wang Changli (2022), incorporating findings from the neoSCORE study, demonstrated that a three-cycle regimen combining immunotherapy and chemotherapy achieved superior primary pathological response rates compared to the two-cycle group. Extending treatment cycles neither increased surgical risks nor postoperative complication rates, while longer treatment durations correlated with higher pathological response rates. Currently, most Phase III studies adopting preoperative neoadjuvant immunotherapy combined with chemotherapy employ three-or four-cycle regimens. Regarding surgical timing, Xu Yuanyuan (2024) synthesized multiple studies to recommend performing surgery approximately one month after the final dose of neoadjuvant immunotherapy, as prolonged intervals may induce fibrosis that elevates surgical difficulty and risks. Hong Wenyan (2024) similarly highlighted the increased incidence of hilar fibrosis following neoadjuvant immunotherapy, leading to a higher proportion of thoracoscopic-to-open thoracotomy transitions and placing greater demands on surgical expertise and experience.

4.2. Controversies in adjuvant therapy decision-making for pCR patients

Whether to initiate subsequent adjuvant therapy for patients achieving pathological complete response (PCR) after neoadjuvant therapy has become a central issue in clinical discussions ^[7]. Liu Xuehui (2025) systematically reviewed relevant controversies, noting that patients achieving PCR in the NADIM study demonstrated superior progression-free survival outcomes. Long-term follow-up data from the CheckMate816 study also corroborated that the overall survival rate in the PCR group was superior to that in the non-PCR group, suggesting potential exemption from subsequent adjuvant therapy for these patients. In contrast, the KEYNOTE-671 study observed that the survival benefit in the perioperative immunotherapy group was not influenced by the status of PCR ^[8]. Wang Chenming (2025), based on a meta-analysis of neoadjuvant chemotherapy phases, proposed that patients achieving PCR still carry certain mortality risks and that PCR should not be equated with disease cure. In clinical practice, individualized treatment plans are often formulated in conjunction with circulating tumor DNA (ctDNA) residual lesion detection results.

4.3. Comparison of safety among different treatment modalities

The overall safety of perioperative immunotherapy is controllable, with variations in the spectrum of adverse reactions across different modalities. Wang Changli (2022) reported that in the CheckMate816 study, the incidence of serious treatment-related adverse events was comparable between neoadjuvant immunotherapy combined with chemotherapy and chemotherapy alone. Hong Wenyan (2024) demonstrated that in the KEYNOTE-671 trial, the incidence of serious adverse events was slightly higher in the pembrolizumab group compared to the placebo group, yet remained within a manageable range. Fang Yujia (2024) noted that the mortality rate of perioperative immunotherapy was slightly higher than that of chemotherapy alone, underscoring the importance of identifying appropriate patient populations for benefit. Xu Yuanyuan (2024) analyzed the impact of neoadjuvant immunotherapy on surgical outcomes, indicating that severe adverse reactions may delay surgical timing, necessitate surgical modality conversion, or even result in missed surgical opportunities. Ni Jun (2021) emphasized the critical importance of standardized baseline assessment and continuous monitoring in clinical practice recommendations.

5. Management strategies for immune-related adverse reactions during the perioperative period

5.1. Prevention and baseline assessment of irAEs

The prevention of immune-related adverse reactions relies on comprehensive evaluation before treatment initiation. Ni Jun (2021) explicitly outlined in relevant clinical guidelines that baseline assessment should encompass multiple components, including medical history collection, physical examination, laboratory testing, and imaging screening. Special populations such as those with autoimmune diseases, organ transplantation history, or chronic viral infections require careful benefit-risk assessment. Endocrine-related indicators, including thyroid function, adrenal function, and pituitary function, must be evaluated before treatment commencement. Pulmonary function tests and cardiac function assessments can assist in predicting perioperative potential risks. Patients should be informed about possible adverse reactions to immunotherapy before treatment initiation and establish a protocol for timely symptom reporting.

5.2. Graded diagnosis and treatment principles for common irAEs

The hierarchical diagnosis and treatment of immune-related adverse reactions form the core of standardized management. According to Ni Jun's (2021) clinical recommendations referencing domestic and international guidelines, adverse reactions are classified into four grades with corresponding management strategies: Grade I reactions require no suspension of immunotherapy but should be closely monitored; Grade II necessitates treatment suspension with consideration of local or systemic medium-dose glucocorticoids^[9]; Grades III and above demand hospitalization with high-dose systemic glucocorticoids, followed by gradual dose reduction after symptom relief. The total treatment course typically lasts several weeks. During glucocorticoid therapy, vigilance is required for complications such as opportunistic infections and osteoporosis. For refractory cases unresponsive to glucocorticoid therapy, immunomodulators like infliximab or tocilizumab may be added.

6. Conclusion

Perioperative immunotherapy for non-small cell lung cancer has evolved from exploratory approaches to established practice, with three modalities, neoadjuvant therapy, adjuvant therapy, and comprehensive therapy, each supported by evidence-based rationale and applicable scenarios. The comprehensive "sandwich" model has demonstrated superior event-free survival benefits in multiple studies, though overall survival data require long-term follow-up validation. Key strategies for maximizing perioperative immunotherapy efficacy include precise identification of beneficiary populations, optimization of treatment cycles and surgical timing, and rational management of immune-related adverse reactions.

Disclosure statement

The authors declare no conflict of interest.

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A Review of Compound Sihuang External Lotion

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Abstract: As a traditional Chinese medicine preparation, Compound Sihuang External Lotion shows unique advantages in the treatment of various clinical diseases, especially in infectious wounds, gouty ulcers, acute gouty arthritis, postoperative care of finger replantation, and contact dermatitis caused by PICC catheterization, which has attracted extensive attention. Since 2011, a lot of studies show that Compound Sihuang External Lotion can improve wound conditions, promote tissue growth, reduce pain and swelling, and significantly lower inflammatory markers like CRP, IL-6, and TNF- α , which improves therapeutic efficacy. Moreover, combining with other therapies like bloodletting puncture, auricular point pressing with beans, and vacuum sealing drainage (VSD), the Compound Sihuang External Lotion further improves its efficacy in wound repair and inflammation control. But even though there was a lot of progress in clinical application, we still need to do more research on its specific mechanism of action, optimal concentration, and dosage form modification to give a better scientific and rational basis for clinical use. This review seeks to cover all the application effects and possible mechanisms of Compound Sihuang External Lotion in treating different diseases, giving references for future research and clinical practice.

Keywords: Compound Sihuang External Lotion; Clinical application; Wound repair

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1. Introduction

Compound Sihuang External Lotion, this a traditional Chinese medicine preparation, it shows a lot of therapeutic potential in treating various clinical conditions, especially those with inflammation, infection, and tissue repair. Its applications encompass the management of gouty ulcers, acute gouty arthritis, orthopedic infections, diabetic foot ulcers, and PICC-related contact dermatitis. Although it has been widely used clinically, the mechanisms behind its efficacy are not fully understood. This review seeks to comprehensively summarize the clinical evidence, including the antibacterial effects, wound healing properties, and advancements in quality control for Compound Sihuang External Lotion.

2. Application of Compound Sihuang External Lotion in the treatment of gouty ulcers

Compound Sihuang External Lotion is a traditional Chinese medicine preparation, it has a good efficacy in treating gouty ulcers. Gouty ulcers are due to the destruction of soft tissues and bones near joints, which is caused by long-term hyperuricemia, and often have severe inflammatory reactions. Compound Sihuang External Lotion has effects of clearing heat and detoxifying, reducing swelling, and relieving pain. It is widely used in the management of gouty ulcers.

Cai Xiaomin et al. (2024) conducted a clinical study on the efficacy of a wet compress formulation combined with Compound Sihuang External Lotion and bloodletting puncture for the treatment of gouty ulcers. A total of 100 patients with gouty ulcers were randomly divided into observation group and control group. The observation group received additional bloodletting puncture on the basis of wet compress with Compound Sihuang External Lotion. The results showed that the total effective rate of the observation group was significantly higher than that of the control group ($P=0.008$), and the levels of CRP and IL-6 in both groups decreased after treatment, with a more significant reduction in the observation group ^[1]. In another study, Chen Haihong et al. (2018) evaluated the effect of external application of Compound Sihuang External Lotion on acute gouty arthritis. Seventy-two patients with acute gouty arthritis were assigned to study group and control group. The study group received Compound Sihuang External Lotion in addition to conventional treatment. The total effective rate of the study group was 83.3%, significantly higher than that of the control group. Both studies confirmed the effectiveness of Compound Sihuang External Lotion in the treatment of gouty ulcers ^[2].

Compound Sihuang External Lotion achieves significant efficacy in treating gouty ulcers, effectively relieving inflammatory responses and improving therapeutic outcomes. Future research may further explore its specific mechanism of action to provide more scientific evidence for clinical application.

3. Compound Sihuang External Lotion has antibacterial effect, and the mechanism behind this effect is not explicitly stated

Compound Sihuang External Lotion isn't just doing well in gouty ulcers, it also has a remarkable antibacterial activity. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common bacterium that is resistant to multiple antibiotics. This bacterium is particularly difficult to treat in clinical settings. Compound Sihuang External Lotion has multiple Chinese herbal components, so it can effectively inhibit MRSA growth. This a new approach for clinical therapy.

Chen Xiaojian (2017) determined the in vitro antibacterial effects of Compound Sihuang External Lotion and its single herbs against MRSA using the Kirby-Bauer (K-B) disk diffusion method. The results showed that Compound Sihuang External Lotion inhibited MRSA growth at a low concentration, with an inhibition zone diameter of up to 15 mm at a high concentration ^[3]. Furthermore, Chen Xiaojian et al. (2017) explored the antibacterial mechanism of Compound Sihuang External Lotion against MRSA and found that it could reduce the activity of alkaline phosphatase (ALP) and cause the leakage of potassium and magnesium ions in MRSA suspension ^[4].

These results show that Compound Sihuang External Lotion has a lot of antibacterial effects via few mechanisms.

Compound Sihuang External Lotion has a very good antibacterial activity, especially for MRSA. Its

multi-component synergistic mechanism offers a novel approach to the treatment of multidrug-resistant bacterial infections. Future studies might further investigate its antibacterial mechanism to enable it to be applied more widely.

4. Compound Sihuang External Lotion in wound repair

Compound Sihuang External Lotion has a good effect on wound repair. Orthopedically infected wounds are a clinical challenge, since conventional therapies usually don't work well and have high recurrence rates. Compound Sihuang External Lotion with vacuum sealing drainage (VSD) can effectively remove wound infection, promote granulation tissue growth, and accelerate wound healing.

Li Xusong et al. (2016) explored the effect of Compound Sihuang External Lotion combined with VSD on the repair of orthopedic infected and defective wounds.

Ninety patients with orthopedically infected wounds were randomly divided into three groups (Groups A, B, and C). Group A received continuous perfusion and irrigation with Compound Sihuang External Lotion combined with VSD; Group B received external application of Compound Sihuang External Lotion alone; Group C received continuous VSD alone. The results revealed that the growth of granulation tissue in Group A was significantly better than that in Groups B and C, and the levels of IL-6 and TNF- α in wound exudate were significantly reduced ^[5-7]. Similar results were reported by Liu Sijing et al. (2016), confirming the excellent performance of Compound Sihuang External Lotion combined with VSD in wound repair ^[8].

Compound Sihuang External Lotion combined with VSD has prominent advantages in the treatment of orthopedic infected wounds, effectively promoting wound healing and reducing infection. Future research may further optimize the administration method of Compound Sihuang External Lotion to enhance its clinical efficacy.

5. Application of Compound Sihuang External Lotion in the treatment of other diseases

Besides gouty ulcers, antibacterial effects, and wound repair, Compound Sihuang External Lotion also shows favorable efficacy in the treatment of other diseases, such as diabetic foot ulcers and contact dermatitis caused by PICC catheterization, further verifying its versatility and clinical value.

Liu Yingchun (2011) investigated the effect of Compound Sihuang External Lotion combined with moxibustion on diabetic foot ulcers. Fifty-two diabetic foot patients were randomly divided into an observation group and a control group. The observation group received Compound Sihuang External Lotion combined with moxibustion, while the control group received Compound Sihuang External Lotion alone. The efficacy of the observation group was significantly better than that of the control group, with a markedly shortened wound healing time ^[9]. In another study, Sun Tingting et al. (2019) evaluated the application effect of wet compress with Compound Sihuang External Lotion on PICC-related contact dermatitis. Forty-eight patients with chronic tumors were randomly divided into a control group and an observation group. The observation group received a wet compress with Compound Sihuang External Lotion on the basis of routine care ^[10]. The results showed that the therapeutic effect of the observation group was significantly better, with obviously reduced dressing changes, treatment duration, and hospitalization costs.

Compound Sihuang External Lotion achieves excellent efficacy in the treatment of various diseases,

relieving symptoms and improving therapeutic outcomes. Future research may expand its application scope to provide more scientific evidence for wider clinical use.

6. Quality control and dosage optimization is the process of improving the quality of a product

To ensure the clinical efficacy of Compound Sihuang External Lotion, research on its quality control and dosage form optimization is particularly important. As a Chinese medicine preparation with complex components, Compound Sihuang External Lotion is difficult to control in quality. The establishment of scientific quality standards and dosage form optimization can improve its stability and effectiveness, ensuring safety and reliability in clinical application.

Yuan Yiming (2017) optimized the extraction process of Compound Sihuang External Lotion by orthogonal design and established an HPLC fingerprint analysis method, providing a reliable basis for scientific evaluation and effective quality control ^[11,12]. The optimized extraction process significantly increased the contents of emodin and berberine hydrochloride in Compound Sihuang External Lotion. In another study, Yuan Yiming et al. (2024) explored the formulation optimization and antibacterial effect of Compound Sihuang Double Hydrogel. Using an orthogonal test, with an artificial skin membrane as the transdermal medium, the forming process of Compound Sihuang Double Hydrogel was optimized through an in vitro transdermal test and antibacterial experiment. The optimized Compound Sihuang Double Hydrogel showed better transdermal effect and stronger antibacterial activity ^[13].

Quality control and dosage form optimization of Compound Sihuang External Lotion can significantly improve its clinical efficacy. Future research may further improve its quality standards and develop more novel dosage forms to provide technical support for its wider application.

7. Conclusion

As a traditional Chinese medicine preparation, Compound Sihuang External Lotion presents significant efficacy in various clinical applications. Studies have shown that it can effectively treat gouty ulcers, acute gouty arthritis and other diseases, and significantly reduce inflammatory markers including CRP, IL-6 and TNF- α . Moreover, combined with other therapies such as bloodletting puncture, auricular point pressing with beans, and VSD, it can further enhance therapeutic effects, accelerate wound repair, and alleviate pain and swelling. These findings provide a solid theoretical basis for the clinical application of Compound Sihuang External Lotion.

Although existing studies have verified the favorable clinical efficacy of Compound Sihuang External Lotion, further research is required to explore its mechanism of action, especially the specific mechanism at the molecular level. In addition, issues such as the optimal concentration, long-term safety, and drug interactions of Compound Sihuang External Lotion deserve in-depth investigation ^[14]. Future studies should also focus on its application in more diseases, such as diabetic foot ulcers and skin reactions induced by radiotherapy for nasopharyngeal carcinoma, to expand its clinical scope and offer more treatment options for patients ^[15].

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Disclosure statement

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Research Progress on Optimization Strategies for Perioperative Accelerated Recovery Nursing Pathway in Lung Cancer

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Abstract: Focusing on the improvement of perioperative accelerated recovery nursing pathways for lung cancer, this study reviews existing literature. The standardized nursing pathway established through interdisciplinary collaboration can shorten postoperative mobilization timing and overall hospitalization duration. Patient education videos combined with psychological interventions enhance patient compliance with diagnostic and therapeutic procedures, reducing the need for clinical analgesics. A comprehensive perioperative respiratory prophylaxis and control protocol decreases the incidence of atelectasis. Evidence-based node-based nursing pathways improve implementation coverage and shorten overall hospitalization duration. Comfortable ward environments alleviate patient psychological anxiety, while nursing protocols are adjusted according to minimally invasive surgical characteristics.

Keywords: Lung cancer; Enhanced Recovery After Surgery (ERAS); Perioperative nursing; Nursing pathway; Optimization strategy

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1. Introduction

Lung cancer is the malignant tumor with the highest incidence and mortality rates in China, with surgical intervention being the primary treatment modality. The introduction of Enhanced Recovery After Surgery (ERAS) principles has improved patient outcomes, yet implementation of nursing pathways faces challenges, such as insufficient evidence translation, fragmented measures, and poor compliance. Systematically refining nursing pathways, integrating multidisciplinary collaboration, precise respiratory management, evidence-based standardization, and innovative service models are critical for enhancing rehabilitation quality. This article reviews recent advancements in improvement strategies from dimensions including interdisciplinary

collaboration, health education, respiratory management, complication prevention and control, and minimally invasive adaptation, providing references for continuous clinical pathway optimization.

2. Strategies for multidisciplinary collaboration and improvement of health education nursing pathways

2.1. Development and implementation of an interdisciplinary collaborative nursing model

Li Jingxiao and Yin Jianing (2026) demonstrated that interdisciplinary collaborative accelerated rehabilitation surgery nursing can improve perioperative care quality for lung cancer patients. The core approach involves establishing a multidisciplinary team comprising surgeons, anesthesiologists, respiratory therapists, dietitians, and specialized nurses to break down disciplinary barriers and establish standardized perioperative care pathways. Clinical controlled studies showed that patients in the interdisciplinary group exhibited shorter postoperative first ambulation time, shorter thoracic drainage tube retention duration, and shorter hospital stays compared to the conventional care group, with lower postoperative pain scores and complication rates. Implementing a closed-loop pathway of “preoperative assessment-intraoperative coordination-postoperative rehabilitation” with dedicated ERAS nurses for continuous quality control can reduce omissions and delays in nursing interventions^[1]. Zhang Junye (2020) reported that in thoracoscopic pneumonectomy patients, nurse-led preoperative rehabilitation training combined with comprehensive anesthesia planning by anesthesiologists reduced postoperative pulmonary complication rates from 18.6% to 7.4%^[2].

2.2. Innovative approaches to diversified health education and psychological support

Liu Junxiao et al. (2022) innovatively introduced a patient video-based health education combined with an accelerated rehabilitation surgery nursing model. Traditional verbal education fails to motivate patients effectively. By producing short videos featuring real-life testimonials from recovered patients and preoperatively screening these videos alongside targeted nursing explanations, anxiety scores were reduced, while postoperative early ambulation and effective sputum expectoration compliance were improved^[3]. The study demonstrated that the observation group achieved an 82.5% ambulation compliance rate on postoperative day 1, significantly higher than the control group's 61.3%. Wang Yu (2020) emphasized the embedded value of perioperative psychological nursing in accelerated recovery pathways. Nurses should use standardized psychological assessment scales to identify high-anxiety patients preoperatively and provide individualized cognitive behavioral interventions to help patients confront surgery and build rehabilitation confidence. Data from the study showed that lung cancer patients receiving systematic psychological support experienced approximately 30% fewer postoperative analgesia pump presses, with psychological interventions enhancing patients' self-control over pain and indirectly optimizing analgesia pathways^[4]. Yan Li (2020) proposed refining health education pathways into five time nodes: “admission day – preoperative day – surgical day – postoperative day – discharge day,” each accompanied by illustrated health manuals and QR code video resources. This structured, visualized approach promotes active patient and family participation in nursing decision-making, effectively addressing fragmented information dissemination in traditional nursing practices and emerging as a key strategy for improvement^[5].

3. Perioperative respiratory management and optimization of complication prevention pathways

3.1. Systematization of comprehensive perioperative respiratory management measures

Tian Maosheng et al. (2022) systematically analyzed the clinical significance of perioperative comprehensive respiratory management for rapid recovery after lung cancer surgery, proposing a tripartite management approach: “preoperative airway preparation – intraoperative lung protection – postoperative airway clearance.” Preoperatively, active circulatory breathing technique (ACBT) combined with inspiratory lung volume training was recommended, administered no less than twice daily for 5–7 days; during surgery, anesthesiologists and nurses collaborated to implement lung-protective ventilation strategies; postoperatively, high-frequency chest wall oscillation and sputum expectoration devices were standardizedly used, and patients were guided to perform early deep breathing and coughing exercises. The study demonstrated that implementation of this comprehensive approach reduced the incidence of postoperative atelectasis from 15.2% to 5.8%, with an average hospital stay shortened by 2.4 days^[6]. Zhang Junye (2020) observed similar outcomes in patients undergoing thoroscopic pneumonectomy, emphasizing that nurses should initiate bedside respiratory function exercises within 6 hours postoperatively—using simple methods such as balloon inflation or respiratory trainers—to promote lung re-expansion without increasing patient discomfort. Yan Li (2020) highlighted the combined use of nebulized inhalation and positional drainage, noting that while postoperative analgesia is essential, it may suppress the cough reflex. They recommended administering nebulized inhalation at least four times daily under effective analgesia, combined with the healthy-side lateral decubitus position for drainage to facilitate deep sputum expulsion.

3.2. Application of accelerated rehabilitation nursing in postoperative complication prevention and control

Lin Liling (2023) reported that in the accelerated recovery nursing pathway, risk assessment should be performed 24 hours before surgery, with patients stratified using the Caprini Thrombosis Risk Assessment Scale. Early implementation of triple prophylactic measures, including ankle pump exercises, intermittent pneumatic compression devices, and low-molecular-weight heparin for high-risk patients, could reduce the incidence of postoperative lower extremity deep vein thrombosis from 4.3% to 0.9%. She proposed optimizing the postoperative nausea and vomiting (PONV) management pathway, including preoperative prophylactic antiemetics such as aprepitant, strict postoperative nausea monitoring, and adjunctive non-pharmacological interventions like menthol oil acupoint massage^[7]. Wang Yu (2020) addressed the conflict between incision pain and early ambulation, recommending a multimodal analgesia strategy with regular administration of nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce opioid use. Patients were encouraged to perform ankle flexion-extension and straight leg raises in bed 4–6 hours postoperatively, with assisted ambulation within 12 hours. Clinical practice demonstrated that this pathway advanced the first postoperative flatus expulsion time to 18.5 ± 3.2 hours, significantly faster than the 30.1 ± 5.4 hours observed in the conventional nursing group. Li Jingxiao and Yin Jianing (2026) further highlighted the unique advantages of interdisciplinary collaboration in rapid complication identification and management. During nursing rounds, when oxygen saturation levels were detected to decline, respiratory therapists could immediately initiate bedside airway clearance and consult physicians for fiberoptic bronchoscopy sputum aspiration. This closed-loop response mechanism reduced complication exacerbation risk by over 60%.

4. Evidence application and novel service models in nursing pathway implementation

4.1. Standardization of accelerated recovery nursing pathways based on optimal evidence

Liu Dandan et al. (2022) systematically summarized the best evidence for perioperative accelerated recovery nursing in lung cancer patients. They identified 22 strong recommendations across seven dimensions: preoperative pre-rehabilitation, intraoperative management, early postoperative mobilization, nutritional support, pain management, catheter management, and discharge follow-up. Key recommendations included preoperative smoking cessation for at least 4 weeks, preoperative carbohydrate loading (consumption of 400 mL of 12.5% carbohydrate beverage 2 hours before surgery), intraoperative maintenance of normal body temperature ($> 36^{\circ}\text{C}$), and removal of urinary catheters within 24 hours postoperatively. These findings provide clear evidence for standardizing nursing pathways^[8]. Liu Dandan et al. (2022) similarly highlighted significant gaps between evidence and clinical practice, attributing this to low nurse awareness of evidence, lack of evaluation mechanisms for pathway implementation, and poor interdepartmental collaboration. They emphasized “evidence translation” as a critical component of improvement strategies, recommending the establishment of an ERAS nursing pathway checklist to convert each evidence item into quantifiable nursing tasks, with daily random checks conducted by head nurses. In a study on perioperative application of single-port thoracoscopic surgery in non-small cell lung cancer patients, Ma Kejian and Yang Junfeng (2024) designed a nursing pathway incorporating 18 quality control nodes based on optimal evidence. Results showed that the pathway group achieved a shorter postoperative hospital stay of 5.3 ± 1.2 days compared to 7.8 ± 1.9 days in the control group, with a pathway completion rate of 94.5%^[9].

4.2. Innovative integration of comfortable ward settings and video-based patient education

Che Guowei and Li Hongjuan (2025) proposed the concept of establishing and applying “comfortable wards,” emphasizing that perioperative lung cancer patients require not only physiological rehabilitation but also psychological comfort and environmental support. The core elements of comfortable wards include a family-like ward environment, individualized analgesia and sedation regimens, and diversified comfort nursing measures^[10]. Their research data demonstrated that under the comfortable ward model, patients’ self-rated postoperative anxiety scale (SAS) scores decreased by 41.6%, nighttime sleep duration increased by an average of 1.8 hours, and the frequency of voluntarily requesting analgesics reduced by 52%. This model is deeply integrated with the accelerated recovery nursing pathway, forming a comprehensive “physiological-psychological-environmental” tripartite strategy. Liu Junxiao et al. (2022) suggested that patient video-based health education could serve as a concrete implementation form of psychological support in comfortable wards. They produced rehabilitation story videos featuring patients of different ages and surgical procedures, which were cyclically played on ward television systems, alongside organizing offline patient exchange meetings. Patients generally reported that viewing real-life cases of successful recovery significantly alleviated surgical fear and enhanced tolerance for postoperative pain and discomfort. This peer education model, combined with professional nursing guidance, compared to traditional one-way education, effectively improved patients’ self-efficacy and facilitated the transition of nursing pathway activities from “passive execution” to “active participation.”

4.3. Fine-tuning of perioperative nursing pathways for minimally invasive surgery

Mark Jian and Yang Junfeng (2024) proposed an optimized “load reduction and acceleration” nursing pathway for single-port thoracoscopic surgery in non-small cell lung cancer (NSCLC) patients. The preoperative regimen was modified from mandatory 12-hour fasting to oral clear liquid intake 2 hours before surgery. During the procedure, thoracic drainage tubes were not routinely placed; instead, fine tubes (14F) were used with portable drainage bottles. Postoperatively, patients were encouraged to consume fluids 2 hours after surgery, transition to a liquid diet at 4 hours, and ambulate at 6 hours. The authors emphasized that nurses must master the identification and management of common post-minimally invasive surgery complications. For instance, shoulder traction pain observed in some patients after single-port thoracoscopic surgery could be effectively alleviated through trapezius muscle massage and positional adjustments. Tian Maosheng et al. (2022) noted that minimally invasive surgery patients exhibited better postoperative cough control compared to open-chest surgery patients, but vigilance remained required for “silent hypoxia.” They recommended dynamic monitoring of oxygen saturation using portable pulse oximeters every 4 hours to track post-exertional oxygen levels. These refined adjustments reflect the trend toward transitioning nursing pathways from “one-size-fits-all” approaches to “precision medicine.” Integrating evidence-based applications, innovative models, and minimally invasive adaptation, current peripartum accelerated recovery nursing pathways for lung cancer have evolved into a multi-tiered framework characterized by “evidence-driven approaches, model integration, and surgical procedure customization.” Future high-quality clinical studies are still needed to further validate the long-term efficacy and health economics value of different optimization strategies.

5. Conclusion

The current perioperative accelerated recovery nursing pathway for lung cancer has evolved from isolated measures to a systematic integration, with a well-defined interdisciplinary collaboration mechanism that highlights the pivotal role of nurses in pathway implementation. Patient video feedback and comfortable wards enhance patient engagement, while evidence-based standardized checklists address bottlenecks in evidence translation. Minimally invasive surgical adaptations emphasize the differentiated needs of surgical procedures. Future research should focus on addressing continuous monitoring of pathway adherence, validating the health economics benefits of refined strategies through multicenter data, and advancing the nursing pathway from merely having a pathway available to continuously refining it.

Disclosure statement

The authors declare no conflict of interest.

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Inhaled Microplastics: Emerging Toxicological Mechanisms and Lung Cancer Risk

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Abstract: Global plastic pollution is rising, drawing attention to health risks from respiratory exposure to micro- and nanoplastics (MNPs); at the same time, lung cancer is also the leading cause of cancer-related deaths worldwide. In recent years, many research groups have studied the relationship between MNPs exposure and the development of lung cancer. Inhalation is the main way for MNPs to enter the respiratory system, and the primary response of cells to plastic particles is an increase in oxidative stress. The consequences of MNP exposure mainly include oxidative stress, DNA damage, inflammatory reaction, macrophage polarization and epithelial-mesenchymal transformation (EMT). These physiological processes eventually induce the malignant transformation of normal cells. In summary, this review systematically sorts out and improves the toxicological theory and mechanism of MNPs driving the malignant transformation of tissues, aiming to provide a scientific basis for environmental health risk assessment and lung cancer prevention and control.

Keywords: MPs; NPs; Lung cancer; Tumor microenvironment; Toxicity

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1. Introduction

Plastics possess structural stability and are widely used, resulting in their persistent accumulation in the environment.^[1] Plastics remaining in the environment are decomposed into smaller fragments through ultraviolet irradiation, mechanical wear, hydrolysis and biodegradation, forming tiny MPs particles with a particle size of less than 5 mm and NPs with a particle size range of less than 1 to 100 nm^[2]. Due to the small size of microplastics and nanoparticles, they can enter different entities of the environment, including water, air and soil, and cause serious pollution. Under the action of natural media such as wind and water flow, micro-nanoplastics are widely migrated and diffused in the ecosystem^[3]. Early research on micro-nanoplastics (MNPs) mainly focused on marine ecosystems, and found that wind direction and ocean currents were key drivers of their global distribution^[4]. In recent years, atmospheric microplastic (MNP) pollution has

gradually attracted people's attention, and climate and seasonal changes are the main influences on its spatial and temporal distribution ^[5].

The equal composition of microns and nanoparticles and other pollutants (heavy metals and volatile organic compounds) is suspended particles (PM), and its particle size ranges from 2.5 μm to 10 μm or more ^[6]. Smaller particles have stronger penetration ability and higher toxicity. PM size is 2.5. It has been proven that it can directly damage DNA in lung tissue and induce pro-inflammatory factors, thus inducing or promoting cancer progression. Clinical and experimental studies have proved that the incidence of lung cancer among non-smokers and smokers has increased significantly in urban areas polluted by PM ^[7]. The current high incidence of NSCLC, coupled with the widespread presence of microplastics in inhaled air, naturally raises concerns about its potential role in the pathogenesis of cancer and its clinical significance.

2. Exposure and deposition of MNPs in the respiratory system

The atmosphere enables significant MNP dispersal. Urban monitoring in Guangzhou and Xi'an, China, has revealed MP and NP abundances reaching up to 1.8×10^5 items/ m^3 and 5×10^5 items/ m^3 , respectively, exposing urban populations to chronic, high-concentration MNP inhalation. Indoor MNP concentrations often exceed outdoor levels, primarily driven by fiber shedding from ubiquitous synthetic textiles and everyday clothing ^[8]. An analysis of PM_{2.5} in Indian cities pointed out that the concentration of PET microplastics in the air can reach up to 158 ng/ m^3 , further highlighting the prevalence of respiratory exposure to MNPs ^[9]. After inhalation exposure, MNPs can be deposited in different areas of the respiratory tract. The sedimentation process is mainly mediated by mechanisms such as inertial impact, gravitational settlement, Brown diffusion and interception ^[10]. Specifically, large particles ($> 10 \mu\text{m}$) are mainly deposited in the upper respiratory tract by inertial impact; medium-sized particles are deposited in the bronchioles by gravity when the air flow slows down; while nanoplastics (NPs) are mainly deep and deposited in the alveolar region through Brown diffusion ^[11]. The respiratory system mainly relies on the mucus ciliation (MCC) mechanism to excrete exogenous micro-nanoplastics (MNPs). However, particles with a diameter of $< 2.5 \mu\text{m}$ can often escape this mechanism; when the accumulation of these particles in the lungs exceeds the body's clearance threshold, it may induce lung injury ^[12].

Pauly et al. (1998) ^[13] detected 87% of MNP in lung tissue samples of lung cancer patients for the first time ^[14], among which polypropylene (PP) and polyethylene (PET) are the main polymers in lung parenchyma and BALF ^[12,15]. Plastic industry workers and textile workers are exposed to high concentrations of particles for a long time, and their risk of pneumoconiosis, asthma, lymphocytic bronchitis, interstitial fibrosis, and even lung cancer is significantly increased ^[16].

3. Toxicological mechanisms of MP-induced lung cancer

3.1. Oxidative stress

Oxidative stress is the core trigger for lung toxicity induced by MNPs. Studies have confirmed that MNPs can significantly promote the production of reactive oxygen (ROS) in pulmonary epithelial cells. Take polystyrene microplastics (PS-MPs) as an example. This substance not only destroys the mitochondrial electron transfer chain and interferes with the purine metabolism, resulting in a large accumulation of mitochondrial active oxygen species (mROS) ^[17,18]. It can also deplete antioxidants such as GSH, SOD and

CAT. It weakens the antioxidant defense system of the lungs. In addition, MNPs will also interfere with the Keap1/Nrf2 signaling pathway and inhibit the transposition of Nrf2 to the nucleus ^[19]. This continuous oxidative stress state will further activate the MAPK/ERK and p38 signaling pathways, causing cell cycle disorder, apoptosis escape and abnormal proliferation ^[20]. More seriously, excessive ROS can induce lipid peroxidation and cause serious DNA damage (such as base modification and DNA fragmentation). If these genetic damages are not effectively repaired and continue to accumulate in cells, they will lead to the activation of proto-oncogenes and the inactivation of tumor suppressor genes, eventually promoting the malignant transformation of normal lung epithelial cells, and causing excessive proliferation and aggregation of abnormal cells in the lungs ^[16].

3.2. Inflammatory response

Inflammation is the body's key defense response to harmful stimuli. Studies show that MNPs can trigger an inflammatory cascade by activating the TLR/MyD88/NF- κ B signaling pathway, inducing the release of inflammatory factors such as IL-1 β , IL-6 and TNF- α ^[21], which leads to chronic lung injury. Animal experiments confirmed that long-term exposure to MNPs of PET, PP and other materials will induce the aggregation of macrophages in the lungs of mice, causing granulomatous inflammatory lesions ^[22,23]. This focal inflammation not only directly causes tissue damage, but also recruits medullary inhibitory cells (MDSCs), thus constructing an immune escape microenvironment for the occurrence of tumors. In addition, MNPs can also induce iron death in normal alveoli and bronchial epithelial cells through the HIF-1 α /HO-1 pathway, eventually causing tissue fibrosis reshaping^[24]. For existing tumor-causing cells (such as in vitro A549 lung cancer cell model), internalized polystyrene nanoplastics (PS-NPs) can further upregulate the expression of IL-8 and NF- κ B, enhancing the potential of the tumor itself to promote inflammation and invasion ^[25].

3.3. Immune dysfunction

Alveolar macrophages (AMs) are the main defense force for immune surveillance and early removal of malignant cells in the body's lungs^[26]. Granules with a diameter of 0.5 μ m are easily internalized by pulmonary macrophages after inhalation. Continuous phagocytic accumulation will lead to lysosomal overload, increased lysosomal membrane permeability (LMP) and obstruction of autophagy function ^[27,28]. This process will directly activate NLRP3 inflammatory bodies and trigger cell death (Pyroptosis), releasing damage-related molecular patterns (DAMPs) and IL-1 β and other pro-inflammatory factors. This kind of macrophage death and functional exhaustion caused by overload directly weakens the immune monitoring ability of the lungs, so that abnormal cells with early mutations can escape and be removed. Not only that, but MNPs can also deeply reshape the early cancer-promoting microenvironment and even the late tumor microenvironment (TME) by inducing the phenotypic polarization of living macrophages ^[29]. Specifically, early M1 phenotypic polarization dominates chronic inflammatory damage, and long-term exposure to MNPs such as polystyrene (PS) or polymethyl methacrylate (PMMA) will induce macrophages to transform into the M2 phenotype, making them similar to tumor-related macrophages (TAMs) characteristics ^[26]. These M2-like cells can continuously secrete IL-10 and TGF- β , thus inhibiting T cell activity and promoting local tissue fibrosis and angiogenesis.

3.4. Fibrosis and epithelial-mesenchymal transition (EMT)

Epithelial-interstitial transformation (EMT) is a highly dynamic biological process; in the process, epithelial cells gradually lose their original characteristics and obtain the phenotype of mesenchymal cells, thus playing a key role in the invasion and metastasis of lung cancer. From the molecular mechanism, the TGF- β /Smad2/3 signaling pathway is a classic pathway for MNPs to induce EMT in lung cells^[30,31]. MNPs exposure can activate key transcription factors such as Snail1/2, Twist1 and ZEB1 downstream of the pathway, breaking the original balance of cells. This abnormal EMT driven by transcription factors is manifested by severe down-regulation of the expression of epithelial markers, such as E-cadherin, and significant upregulation of interstitial markers such as waveform protein and α -SMA^[32]. The transformation of phenotype gives cancer cells stronger movement and degradation ability, allowing them to break through the cell basal membrane and enter the blood circulation system, thus accelerating the early invasion and distant metastasis of the tumor^[33].

3.5. Other mechanisms

Micro/nanoplastics (MNPs) can adsorb environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs), heavy metals and phthalates through π - π accumulation and electrostatic action^[34]. In the A549 cell model, it was observed that MNPs induce oxidative stress and genetic toxicity after carrying the attached pollutants into the cell^[35]. In addition, MNPs will also interfere with the microecology of the lungs. Changing the composition of respiratory symbiotic bacteria they will lead to a flora imbalance and aggravate chronic inflammatory reactions. This continuous microecological imbalance will abnormally activate the TLR signaling pathway and change the local tumor microenvironment (TME)^[36].

4. Effects of plastics on signaling pathways in lung cancer cells

Existing studies suggest that combined exposure to micro/nanoplastics (MNPs) and ozone may reduce the response rate of patients with non-small cell lung cancer (NSCLC) to immunotherapy and shorten their recurrence-free survival. Studies show that polystyrene nanoplastics (PS-NPs) are internalized by A549 lung cancer cells, significantly upregulating inflammatory mediators such as IL-8, NF- κ B and TNF- α ^[37]. At the same time, PS-NPs can upregulate the expression of DR5 and caspase-grade apoptotic proteins (such as caspase-3, 8, 9) and cytochrome c, thus inducing cell cycle blockage and mitochondrial dysfunction^[25]. Jin et al. found that polyvinyl chloride (PVC) can also induce the aging of A549 cells through oxidative stress^[38]. In Calu-3 pulmonary epithelial cells with secretory functions, polylactic acid (PLA) nanoparticles will destroy the close connection between cells, reduce mucus secretion and induce DNA damage; at the same time, it will also stimulate the excessive secretion and cell remodeling of related proteins. Interestingly, although it is also a PLA particle, it does not show significant direct lethal cytotoxicity to A549 cells, but the potential metabolic interference effect of this part of the particle still needs to be further clarified^[39].

5. Current research gaps

Although advances have been made in micro/nanoplastics (MNPs) research, critical knowledge gaps remain in this field. Most current experiments adopt standard samples with high concentrations and homogeneous physicochemical properties, neglecting environmental particle heterogeneity and real-world long-term low-

dose human exposure scenarios. The release kinetics of MNPs as carcinogen carriers and their synergistic carcinogenic risks have not been fully quantified. Moreover, technical bottlenecks in nanoparticle detection greatly hinder the clarification of the epidemiological association between MNPs and lung cancer risk.

6. Conclusion and perspectives

Environmental MNPs are significant atmospheric risk factors for lung cancer. Inhaled MNPs accumulate in deep lung tissues. They exert chronic toxicity. They drive cancer progression through multiple pathways. These include oxidative stress-induced genotoxicity. They also involve pro-cancer inflammation, immune evasion, and EMT activation. These processes create a favorable niche for malignant transformation and metastasis. Their “Trojan horse” effect amplifies the toxicity of adsorbed mutagens. Clinical evidence for MNPs is still evolving. Toxicological data confirm their high carcinogenic potential. This highlights the need for multidisciplinary risk assessment. This assessment is essential to safeguard public health.

Disclosure statement

The authors declare no conflict of interest.

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Study on the Impact of the “H2H” Nutritional Management Model Based on the Internet Platform on the Nutritional Status and Quality of Life of Patients after Esophageal Cancer Surgery

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Abstract: *Objective:* To explore the influence of the “H2H” (Hospital to Home) nutrition management model based on the Internet platform on the nutritional status and quality of life of patients after esophageal cancer surgery, and to provide evidence-based evidence for the continuous nutrition management after esophageal cancer surgery. *Methods:* From August 2025 to April 2026, 138 postoperative patients with esophageal cancer in our hospital were randomly divided into a control group of 71 cases and an observation group of 67 cases by convenient sampling method. The control group received conventional nutritional management, while the observation group implemented the “H2H” nutritional management model based on the Internet platform on this basis. The intervention period was 3 months after discharge. The nutritional indicators, the score of Nutritional Risk Screening 2002 (NRS2002) and the score of the Quality of Life Questionnaire for Cancer Patients (QLQ-C30) of the two groups of patients before the intervention (1 day before discharge) and after the intervention (3 months after discharge) were compared. *Results:* Before the intervention, there were no statistically significant differences in various nutritional indicators, NRS2002 scores and scores of each functional domain of QLQ-C30 between the two groups of patients ($P > 0.05$). After the intervention, the levels of body weight, prealbumin, serum albumin and hemoglobin in the observation group were significantly higher than those in the control group ($P < 0.05$), and the standardized scores of physical, cognitive, emotional, social and role functions in the QLQ-C30 scale were significantly higher than those in the control group ($P < 0.05$). There was no statistically significant difference in transferrin levels and NRS2002 scores between the two groups ($P > 0.05$). *Conclusion:* The “H2H” nutrition management model based on the Internet platform can effectively improve the core nutritional indicators of postoperative patients with esophageal cancer, enhance their quality of life, and has high patient compliance. It is an efficient postoperative continuous nutrition management model and is worthy of promotion and application in the field of clinical tumor care.

Keywords: Esophageal cancer; Internet; “H2H” nutritional management model; Quality of life; Nutritional indicators

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1. Introduction

Esophageal cancer is one of the common malignant tumors that threatens the life and health of Chinese residents ^[1,2], and its incidence ranks 8th in the world ^[3]. Due to disease consumption, surgical reconstruction of the digestive tract, and adverse reactions of radiotherapy and chemotherapy, patients with esophageal cancer bear a huge nutritional burden during and after treatment. The incidence of malnutrition is as high as 60% to 85%, ranking first among all malignant tumors, and has been proven to be an independent risk factor for complications and prognosis ^[4]. Therefore, establishing a comprehensive and personalized nutritional management model is crucial to improving the clinical outcomes of postoperative patients with esophageal cancer. Routine nutritional management is mostly limited to the patient’s hospitalization period, and nutrition guidance is mostly provided by short-term telephone follow-up after discharge. There are problems such as management gaps, lack of personalized guidance, and insufficient supervision of patients’ performance, making it difficult to meet the nutritional needs of patients for long-term home rehabilitation ^[5]. In recent years, with the rapid development of “Internet + nursing services”, the Internet platform has provided technical support for continuous nutrition management outside the hospital, which can break the time and space constraints to achieve real-time monitoring, online guidance and dynamic follow-up, thereby improving patients’ awareness and compliance of health management and meeting the personalized health needs of patients at different levels ^[6]. The H2H (Hospital to Home) nutrition management model was first proposed in China in 2016 by the Clinical Nutrition Department of West China Hospital of Sichuan University ^[7]. The core is to establish a full-range nutrition management path from hospital to home. Seamless nutrition support is achieved through in-hospital nutritional screening and treatment, development of personalized family meal plans, and continuous follow-up via phone/WeChat/App. Research shows that this model effectively improves the nutritional status of patients (especially patients with chronic diseases), significantly reduces the incidence of complications and re-hospitalization rates, and improves treatment compliance and quality of life ^[8]. It extends traditional nutritional intervention limited to hospitalization into a long-term, dynamic, full-course health management. Therefore, this study uses the Internet platform to expand the “H2H” nutritional management model to patients after esophageal cancer surgery, build a standardized intervention process, explore its impact on patients’ nutritional status and quality of life, and provide a reference for optimizing the nutritional management model after esophageal cancer surgery.

2. Materials and methods

2.1. Research objects

A convenience sampling method was used to select esophageal cancer surgery patients who visited a cancer specialist hospital in Guangzhou from August 2025 to April 2026 as the research subjects.

Inclusion criteria: (1) meet the diagnostic criteria for esophageal cancer in the “Standards for the Diagnosis and Treatment of Esophageal Cancer (2018 Edition)” ^[9]; (2) undergo elective radical resection

for esophageal cancer; (3) Karnofsky functional status score (Karnofsky, KPS) ≥ 60 points; (4) estimated survival time is more than 3 months; (5) normal language communication ability; (6) able to use smartphones independently to cooperate with questionnaires; (7) informed consent and cooperation with follow-up.

Exclusion criteria: (1) distant metastasis of tumors; (2) multiple organ failure; (3) severe lesions in the lungs and pleura before surgery; (4) combined with metabolic diseases such as diabetes; (5) receiving immunotherapy; (6) severe cognitive dysfunction.

Taking serum albumin as the main outcome indicator, referring to previous studies^[10], the mean difference in serum albumin between the experimental group and the control group after intervention was $\delta = 3.3$ g/L, and the standard deviation was 4.8. It was assumed that two-sided $\alpha = 0.05$, and the test power was $1 - \beta = 0.90$. Using the sample size calculation formula for comparing the means of the two groups, the minimum sample size for each group was calculated to be 62 cases. Considering the 20% loss to follow-up rate, 75 cases were included in each group. During the course of the study, 1 case died in the control group, 3 cases received immunotherapy, 2 cases died in the observation group, 1 case suffered multiple organ failure, and 5 cases received immunotherapy. The final loss to follow-up rate was 8%. A total of 138 valid samples were included: 71 cases in the control group and 67 cases in the observation group.

This study was approved by the Medical Ethics Committee of Sun Yat-sen University Cancer Center, and all subjects signed informed consent. This study was approved by the Ethics Committee of the Clinical Medical Research Center of our hospital (Approval No. B2025-669-01).

2.2. Intervention methods

2.2.1. Control group

Standardized routine nutrition management after esophageal cancer surgery is implemented, which is completed by nutrition specialist nurses and esophageal cancer specialists: (1) nutritional risk screening using the NRS2002 scale within 24 hours of admission, one-on-one nutrition education and personalized dietary guidance, and explanation of dietary principles, food selection and energy intake points after esophageal cancer surgery; (2) preoperative assessment of the patient's nutritional status, based on The NRS2002 score provides targeted nutritional support such as oral nutritional supplements, enteral nutrition, and intravenous nutrition; (3) a paper nutrition guidance manual is issued upon discharge to inform the patient of home nutrition precautions, review time and items; (4) telephone follow-up is conducted every 2 weeks after discharge, with each follow-up lasting 10 to 15 minutes. The content includes diet implementation, weight changes, symptoms of discomfort, etc. Targeted nutritional guidance is provided and the patient is reminded to return to the hospital for review on time.

2.2.2. Observation team

On the basis of the control group, "Internet +" H2H nutrition management was implemented. A "H2H" nutritional management team was established, consisting of a head nurse, an esophageal cancer specialist, and two nutrition specialist nurses. All members have received professional training in tumor nutrition management, Internet platform operation, etc., and can only participate in the study if they pass the assessment with a 100% passing rate. Relying on the hospital's "Internet + Nursing" service platform and self-developed mini-programs, the intervention period is 3 months after discharge.

2.2.2.1. In-hospital nutrition management

- (1) Strengthening nutrition education: In addition to conventional guidance, patients and primary caregivers are provided with intensive training on dietary transition principles, energy and food estimation methods after esophageal cancer surgery using teaching aids such as food models and energy calculation turntables.
- (2) Develop a personalized nutrition plan: 1 day before discharge, doctors and nutrition specialist nurses will jointly develop an individualized family nutrition treatment plan based on the patient's nutritional risk level, energy needs and digestive tract function, and clarify the nutritional support path (oral, tube feeding or combined), the type and dosage of nutritional preparations, and the diet transition plan.
- (3) Platform usage training: Provide one-on-one guidance to patients and caregivers to master various functions of the "Esophageal e-Nutrition" applet to ensure that they can independently complete information entry, course learning, online consultation and other operations.

2.2.2.2. Home nutrition management

Relying on the nutrition management applet to realize the management of four core functional modules, the nutrition specialist nurse is responsible for the maintenance and operation:

- (1) Online education: According to the family enteral nutrition guide, nine standardized teaching videos are filmed, covering the knowledge and skills that must be mastered in family nutrition such as nutrient solution preparation, bolus injection, use of nutrition pumps, home care of nutrition tubes, common complications and their treatment. After patients or caregivers complete all courses and pass the online assessment, they can obtain electronic certificates to ensure that nutrition knowledge is in place;
- (2) Online Consultation: A 24-hour online consultation module is set up. Patients can raise nutritional issues during home health care at any time, and nutrition specialist nurses will reply within 12 hours. Questions that appear frequently on the platform (such as diet transition, weight loss treatment) are sorted out, summarized, and made a special push for all patients to refer to;
- (3) Nutritional monitoring: The platform design embeds commonly used nutrients and food ingredients and energy selection modules. Patients check and enter their daily diet, and the mini program automatically calculates whether the nutritional intake meets the target needs. For patients with insufficient intake, personalized nutritional supplement recommendations are pushed in real time; patients measure and upload their weight once a week at a fixed time, and if the weight drops by >5% or continues to drop compared with discharge, the system automatically warns, and the nutrition management team immediately intervenes for review;
- (4) Program supervision and offline support: If the patient fails to check in for nutrition for 3 consecutive days, the system automatically triggers a reminder, and the management team conducts a follow-up phone call to understand the reasons and assist in solving the problem. Patients who need home care can make an appointment through the "Online Nurse Appointment" function of the hospital APP, and a nutrition specialist nurse will come to provide nutrition tube maintenance and other services.

2.2.2.3. Outpatient follow-up

Nutrition specialist nurses conduct telephone follow-up visits once a week, with each follow-up lasting 15 to 20 minutes. The content includes the implementation of the nutrition plan, the patient's stress state, treatment compliance and psychological state, etc.; combined with objective data such as laboratory indicators and

body composition analysis reports from the nutrition specialist clinic when the patient returns to the hospital for follow-up visits, personalized and dynamic nutrition plan adjustments are made for the patient to ensure the pertinence and effectiveness of nutrition management until the end of the intervention cycle.

2.3. Observation indicators

2.3.1. Nutritional indicators

Collect nutritional indicators related to patients' morning fasting, including: (1) Body weight/kg; (2) Prealbumin (PA): The normal value of PA is 25–40 mg/dL, which reflects the short-term nutritional status and has high sensitivity; (3) Serum albumin (albumin, ALB): The normal value of ALB is 40–55 g/L, which reflects the body's long-term nutritional reserve; (4) Hemoglobin (HGB): normal value for men is 130–175 g/L, and for women is 115–150 g/L; (5) Transferrin (g/L): normal value is 2.0–4.0 g/L, assisting in assessing nutritional status. On the day of enrollment, the patients' fasting venous blood was collected uniformly, and the blood samples were sent to the Laboratory Department of our hospital for laboratory biochemical index testing. For re-examination after the patient left the hospital (90 days after the intervention), the fasting venous blood was collected by the blood collection center of the outpatient department of our hospital, and the blood samples were sent to the Laboratory Department for laboratory biochemical index testing.

2.3.2. Nutritional Risk Screening 2002 (NRS2002)

NRS2002 is the preferred tool ^[11] for nutritional risk assessment of inpatients, with good reliability and validity (Cronbach's α coefficient is 0.82–0.86). The scale includes nutritional status score (0–3 points), disease severity score (0–3 points), and age-adjusted score (≥ 70 years old plus 1 point). A total score of < 3 points indicates no nutritional risk, and nutritional risk screening is only performed once a week; a total score of ≥ 3 points indicates the existence of nutritional risk, and timely nutritional support intervention is required. Members of the research team will evaluate the patients on the day of enrollment and 90 days after the intervention.

2.3.3. Quality of life

Use the Quality of Life Questionnaire-Core 30 (QLQ-C30) for cancer patients to evaluate the quality of life of patients before and after intervention ^[12]. This scale is widely used to assess the quality of life of patients with various types of tumors. Its reliability and validity have been verified by many domestic studies, and it is suitable for patients after esophageal cancer surgery. The scale contains 15 domains with a total of 30 items. This study focuses on evaluating its 5 functional domains, namely physical function (PF), role function (RF), cognitive function (CF), emotional function (EF) and social function (SF). There are 15 items, each of which has a score ranging from 1 to 4 points. The rough score is equal to the total score of the items in each field divided by the number of items. The extreme difference method is used to convert the rough score into a standardized score (0 to 100 points). The higher the score, the better the quality of life. The Cronbach's alpha coefficient of the scale is 0.885, indicating good internal reliability and validity. Members of the research team will evaluate the patients on the day of enrollment and 90 days after the intervention.

2.4. Data collection and quality control

Before the investigation began, the project was approved by the hospital ethics committee and the hospital nursing department provided consent. Team members were uniformly trained and assessed. Only those

who passed the training could participate in the study. The same group of specialist nurses conducted all interventions. During the investigation process, unified instructions were used to explain the purpose and significance of the study to the respondents, solicit the patient's consent and understanding, and explain the filling instructions in detail. The intervention process is regularly supervised and inspected. The head nurse organizes an intervention quality inspection every month, establishes a follow-up quality control mechanism, and conducts random checks on follow-up records (spot check rate $\geq 20\%$) to ensure that the follow-up information is true and complete. After the survey is completed, check whether there are any errors or omissions, confirm in a timely manner, and collect the questionnaire after confirmation.

2.5. Statistical methods

Two trained researchers will enter the data in pairs. After the entry is completed, cross-checking will be carried out. Abnormal data will be verified and corrected in a timely manner. SPSS 26.0 software was used for data processing. If the measurement data conformed to the normal distribution, it was expressed as mean \pm standard deviation (SD). If it did not conform to the normal distribution, it was expressed as the median M. The count data was expressed as the rate (%). The t-test and χ^2 test were used to compare the differences between groups. $P < 0.05$ was considered a statistically significant difference.

3. Results

3.1. Comparison of general information between the two groups of esophageal cancer patients

Before intervention, there were no statistically significant differences between the two groups of patients in terms of gender, age, lesion location, tumor type and other general information ($P > 0.05$). See **Table 1** for details.

Table 1. Comparison of general information between the two groups of patients after surgery for esophageal cancer

Characteristics	Control group ($n = 71$)	Observation group ($n = 67$)	Test statistic	<i>P</i> value
Gender, n (%)				
Male	52	53	$\chi^2 = 0.953$	0.329
Female	19	13		
Age (years), mean \pm SD	64.93 \pm 8.26	63.38 \pm 7.55	$t = -1.145$	0.254
Lesion location, n (%)				
Upper thoracic esophagus	5	9	$\chi^2 = 0.198$	0.884
Middle thoracic esophagus	39	35		
Lower thoracic esophagus	27	23		
Tumor type, n (%)				
Adenocarcinoma	36	32	$\chi^2 = 0.953$	0.923
Squamous cell carcinoma	24	23		
Adenosquamous carcinoma	11	12		

3.2. Comparison of nutritional indicators and NRS2002 scores between the two groups of esophageal cancer patients before and after intervention

Before the intervention, there was no statistically significant difference in the nutritional indicators (body weight, prealbumin, serum albumin, hemoglobin, transferrin) and NRS2002 scores between the two groups of patients ($P > 0.05$); after the intervention, the observation group's weight, prealbumin, and The levels of albumin, serum albumin, and hemoglobin were significantly higher than those in the control group, and the difference was statistically significant ($P < 0.05$); when comparing the transferrin levels and NRS2002 scores between the two groups, the difference was not statistically significant ($P > 0.05$). See **Table 2** for details.

Table 2. Comparison of nutritional indicators and NRS2002 scores between two groups of patients after esophageal cancer surgery before and after intervention

Indicators	Group	Before intervention	After intervention	Intergroup <i>t</i> -value (after intervention)	<i>P</i> value (after intervention)
Body weight (kg)	Control	58.39 ± 10.10	57.58 ± 9.81	-2.003	0.049
	Observation	61.42 ± 10.10	61.05 ± 10.20		
NRS2002 score (points)	Control	2.93 ± 0.72	2.91 ± 0.68	0.230	0.410
	Observation	2.86 ± 0.47	2.79 ± 0.62		
Prealbumin (mg/dL)	Control	24.69 ± 4.80	22.50 ± 4.47	-2.751	0.033
	Observation	23.27 ± 4.48	26.11 ± 4.48		
Serum albumin (g/L)	Control	42.79 ± 5.24	44.23 ± 4.61	-2.239	0.024
	Observation	43.28 ± 4.74	46.85 ± 5.37		
Transferrin (g/L)	Control	2.77 ± 0.52	2.70 ± 0.55	2.920	0.250
	Observation	2.18 ± 0.48	2.44 ± 0.50		
Hemoglobin (g/L)	Control	106.70 ± 8.52	107.43 ± 11.26	-2.712	0.007
	Observation	105.54 ± 9.51	109.92 ± 11.35		

3.2. Comparison of quality of life between the two groups of esophageal cancer patients before and after intervention

Before the intervention, there was no statistically significant difference in the scores in each functional domain of the QLQ-C30 scale between the two groups of patients ($P > 0.05$); after the intervention, the scores in each functional domain of the two groups were significantly higher than before the intervention ($P < 0.05$), and the scores of physical function, cognitive function, emotional function, social function, and role function in the observation group were significantly higher than those in the control group, and the difference was statistically significant ($P < 0.05$). See **Table 3** for details.

Table 3. Comparison of quality of life between two groups of patients after esophageal cancer surgery before and after intervention (minutes, mean ± SD)

Functional domains	Group	Before intervention	After intervention	Intergroup <i>t</i> -value (after intervention)	<i>P</i> value (after intervention)
Physical function	Control	47.25 ± 9.35	51.21 ± 8.19	0.631	0.008
	Observation	47.87 ± 9.18	54.03 ± 8.21		
Cognitive function	Control	51.16 ± 6.74	56.22 ± 5.35	3.071	0.003
	Observation	50.67 ± 6.43	62.56 ± 5.15		

Functional domains	Group	Before intervention	After intervention	Intergroup <i>t</i> -value (after intervention)	<i>P</i> value (after intervention)
Emotional function	Control	56.17 ± 7.85	62.73 ± 6.56	-4.213	0.001
	Observation	57.09 ± 7.53	65.01 ± 6.24		
Social function	Control	53.86 ± 6.37	61.26 ± 6.24	-2.916	0.004
	Observation	54.85 ± 6.56	67.63 ± 7.56		
Role function	Control	55.15 ± 4.23	59.71 ± 5.45	-4.356	0.001
	Observation	54.95 ± 3.45	63.05 ± 4.22		

4. Discussion

4.1. The “H2H” nutritional management model based on the Internet platform can effectively improve the nutritional status of esophageal cancer patients

The results of this study show that after the intervention, the core nutritional indicators such as body weight, prealbumin, serum albumin, and hemoglobin in the observation group were significantly higher than those in the control group ($P < 0.05$), suggesting that the “H2H” nutritional management model based on the Internet platform can effectively improve the nutritional status of patients after esophageal cancer surgery. This is consistent with the research conclusions of Liu Jiahuan et al. on gastric cancer patients^[10].

The “H2H” nutrition management model based on the Internet platform has constructed a three-level full nutrition management system of “hospital-home-follow-up”, which effectively makes up for the out-of-hospital disconnection problem of the traditional management model: in-hospital standardized education and personalized plan formulation lay a solid foundation of knowledge and plans for patients’ home nutrition management; the home stage relies on the Internet The platform realizes real-time monitoring of nutritional intake and dynamic tracking of weight, and provides timely intervention for those with insufficient intake and excessive weight loss to ensure the timeliness of nutritional intervention. The convenient management based on the nutrition management applet, combined with the dynamic adjustment plan of follow-up indicators, achieves precise and dynamic nutrition management, effectively avoiding the limitations of a single plan. At the same time, the online education module of the platform improves the practical nutrition skills of patients and caregivers through practical videos. 24-hour online consultation and door-to-door nursing services solve practical difficulties in home rehabilitation, significantly improve the implementation compliance of nutrition plans, and ultimately achieve effective improvements in nutritional status.

In this study, there is no significant difference in the level of transferrin between the two groups, which is presumed to be related to the short intervention period and long-term targeted intervention for postoperative iron absorption disorder in patients with esophageal cancer. The possible reason why there was no significant difference in the NRS2002 score before and after intervention was that the intervention cycle was short and the baseline nutritional risk level of the two groups was similar.

4.2. The “H2H” nutritional management model based on the Internet platform can significantly improve patients’ quality of life

The results of this study show that after the intervention, the scores of physical function, cognitive function, emotional function, social function and role function in the QLQ-C30 scale of the observation group were significantly higher than those of the control group ($P < 0.05$), suggesting that this model can effectively improve the quality of life of patients with esophageal cancer after surgery^[13]. Nutritional status is closely

related to the quality of life of cancer patients. Patients after esophageal cancer surgery are prone to physical symptoms such as fatigue, weight loss, and loss of appetite due to malnutrition, which seriously affects physical functions. At the same time, impaired eating function and poor nutritional status can easily trigger negative emotions such as anxiety and depression, reduce social willingness and role functions, and then lead to a decline in quality of life ^[14]. Comprehensive nutritional management can reduce fatigue and other physical symptoms by improving nutritional status, ensuring the recovery of physical functions; improvement of physical symptoms can alleviate negative emotions, enhance recovery confidence, promote patients to better assume family and social roles, and improve emotional and role functions ^[15]; the self-management module of the Internet platform can enhance patients' sense of control over recovery and improve cognitive functions; online consultation and follow-up interaction can strengthen the doctor-patient relationship, reduce loneliness and helplessness, and improve social functions. However, due to insufficient out-of-hospital intervention, traditional routine nutrition management has limited improvements in patients' nutritional status, physical symptoms, and psychological status, and has poor effects on improving quality of life. The results of this study confirm that comprehensive nutritional management has important clinical significance in improving the quality of life of patients with esophageal cancer after surgery.

4.3. The significance of the “H2H” nutrition management model based on the Internet platform for the continued care of patients after esophageal cancer surgery

The “H2H” nutritional management model based on the Internet platform provides an efficient, practical path for continued care after esophageal cancer surgery. It guides patients to actively participate in nutritional management through online education, real-time monitoring, independent check-in, etc., which not only improves patients' disease self-management capabilities and nutritional knowledge ^[16–19], but also uses 24-hour The active communication model of online consultation and regular online follow-up has enhanced the trust between nurses and patients, alleviated patients' negative emotions, significantly improved the implementation of nutritional plans and overall compliance with treatment, and is in line with the development trend of chronic cancer management. At the same time, this model realizes the deep integration of the Internet platform and extended care, effectively making up for the disconnection of traditional out-of-hospital nutrition management, strengthening the coordination and integration of health care between hospitals and communities, and providing an evidence-based basis for the networked and diversified development of clinical extended care ^[20–22]. It also provides practical ideas for the expanded application of Internet technology in the full-cycle health management of cancer patients. It can further improve the extended care process and provide better and more convenient full-cycle health care services for patients after esophageal cancer surgery.

4.4. Research limitations and clinical application suggestions

This study has certain limitations: first, the sample size of the study is small and it is a single-center study, so the extrapolation of the study results is limited; second, the intervention period is short, and the intervention effect was only observed 3 months after discharge. There was no long-term follow-up of the patients, so the long-term intervention effect of this model cannot be evaluated.

5. Summary

The H2H nutrition management model based on the Internet platform constructed in this study realizes the seamless management of esophageal cancer patients from hospital to home, can effectively improve their nutritional status, and improve their quality of life from multiple dimensions, such as body, role, emotion, cognition, and social function. At the same time, it can also improve patients' nutritional management compliance and self-management ability. It is an efficient and feasible continuous nutritional management model after esophageal cancer surgery. It conforms to the development trend of the "Internet + medical health" era and has high clinical practical value and promotion significance. In the future, randomized controlled clinical studies with large samples, multi-centers, and long-term follow-up can be carried out, and a long-term intervention mechanism can be established to further verify the long-term effects and cost-effectiveness of this model. At the same time, joint intervention plans can be developed with sports rehabilitation specialists, psychotherapists, etc., and the functions of the Internet platform can be continuously expanded to achieve multi-dimensional and full-course rehabilitation management for patients after tumor surgery, and provide more powerful support for the long-term survival and improvement of the quality of life of tumor patients.

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Disclosure statement

The authors declare no conflict of interest.

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Effectiveness and Safety of Macitentan in Hemodialysis Patients with Pulmonary Hypertension: A Retrospective and Observational Study

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Abstract: *Background:* Pulmonary hypertension (PH) is a rare disease often associated with high mortality and is recently recognized as a common complication secondary to chronic kidney disease. Macitentan can improve clinical outcomes in patients with pulmonary artery hypertension in clinical trials. To observe the clinical efficacy and safety of macitentan tablets in the treatment of hemodialysis HD patients complicated with PH. *Methods:* This was a retrospective study performed in the renal division of Shaanxi Provincial People's Hospital between January 1, 2024, and December 31, 2025. A total of 10 patients with PAH who underwent regular hemodialysis (HD) (three times weekly for 4 hours each session) were enrolled. All patients received basic treatment, based on which macitentan tablets were added (10 mg per dose, once daily, oral administration), with a treatment cycle of 12 weeks. The 6-minute walk distance (6MWD), pulmonary artery systolic pressure (PASP) measured by echocardiography, and WHO functional class were compared before and after treatment. Serious adverse events (SAEs) and adverse drug reactions (ADRs) of macitentan were collected. *Results:* After 12 weeks of treatment, the proportion of patients with WHO-FC II increased from 20% to 60% ($p = 0.046$). LVEF showed an upward trend, with the median rising from 50.5% to 53.5% ($p = 0.03$). The mean 6MWD increased by 28.8 m ($p = 0.035$). Median NT-proBNP decreased from 39018 ng/L to 18987.5 ng/L ($p = 0.017$). Meanwhile, mean PASP dropped from 52.1 (5.363) mmHg to 39.4 (8.796) mmHg. Only two mild adverse reactions (dizziness and aggravated mild anemia) occurred during the entire observation period, which were relieved after symptomatic treatment. No severe liver or renal function impairment or treatment discontinuation was observed. *Conclusion:* With regular HD and basic treatment, macitentan demonstrates clear short-term efficacy and controllable safety in HD patients with WHO class II-III PH.

Keywords: Macitentan; Hemodialysis (HD); Pulmonary hypertension (PH)

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1. Introduction

Pulmonary arterial hypertension (PAH) is a chronic and progressive disease characterized by the presence of precapillary pulmonary hypertension^[1,2]. The disease results from functional and structural changes in the pulmonary vasculature, leading to increased pulmonary vascular resistance, right ventricular failure, and subsequently death^[3,4]. In the general population, the prevalence of PAH is estimated to be 15-50 cases per million, with an annual incidence rate of approximately 2.4 cases per million^[5]. However, pulmonary hypertension (PH) affects 21% to 41% of patients with chronic kidney disease (CKD) and up to 60% of patients with kidney failure receiving hemodialysis^[6,7]. Mechanisms responsible for PAH in HD patients have not been completely understood. However, abnormal endothelium-dependent vasodilatation, vascular calcification, thromboembolic disease, hypervolemia, increased pulmonary vascular flow due to the presence of arterio-venous fistulas, anemia and sleep-disordered breathing play a role^[8,9]. The classic symptoms of PAH occur relatively late and may be confounded by overhydration in HD patients, leading to the delayed diagnosis and the worsening of prognosis^[10]. To date, there are no targeted treatments for PH in patients with CKD. Macitentan, as an endothelin receptor antagonist, was approved by the China National Medical Products Administration in 2018 as a monotherapy or combination therapy for the treatment of adult patients with PAH. The approval was mostly based on findings from the global SERAPHIN trial, and the drug is currently indicated for patients with PAH and preserved renal function. However, limited data are available regarding the application of macitentan in patients undergoing hemodialysis. The present study aims to evaluate the effectiveness and safety of macitentan prescribed to patients undergoing hemodialysis complicated with PAH.

2. Methods

2.1. Study design and participants

The retrospective study was performed in the renal division of Shaanxi Provincial People's Hospital between January 1, 2024, and December 31, 2025. A total of 10 patients with PAH who underwent regular hemodialysis (HD) (three times weekly for 4 hours each session) were enrolled. All patients were treated with high-flux dialyzers, with a blood flow of 250–300 mL/min and a dialysate flow of 500 mL/min.

Patients were eligible if they had confirmed by echocardiography (pulmonary artery systolic pressure, PASP ≥ 35 mmHg) and conforming to WHO functional class II-III, were aged 18 years old, had received regular hemodialysis (HD) for ≥ 3 months, and had available data in their medical chart for data collection, with follow-up data available at 3–6 months. All enrolled patients were given basic treatments, including correction of anemia with erythropoietin, iron supplementation, and improvement of heart failure with ACEI/ARB drugs.

Patients with the following conditions were excluded: allergy to macitentan or drug excipients; pregnant, lactating women or those with pregnancy plans; severe liver function impairment (Child-Pugh class C); acute myocardial infarction, cerebral hemorrhage or severe infection occurring within the past 1 month; expected survival time < 6 months.

All patients maintained their original HD regimen and basic treatment. On this basis, Macitentan Tablets were additionally administered (dosage: 10 mg per time, once a day, taken orally). On dialysis days, the medication time should avoid the dialysis process, which is fixed at 2 hours before dialysis or 4 hours after dialysis to prevent drug clearance from affecting the blood drug concentration. The continuous treatment

duration was 12 weeks.

Written informed consent was obtained from all patients. The trial adhered to the Declaration of Helsinki and the research protocol was approved by local institutional review boards or independent ethics committees.

2.2. Clinical and laboratory data collection

Patient data on baseline characteristics (age, gender, medication used), etiology of CKD, laboratory tests and hemodynamics were recorded.

Before treatment, 4 weeks, 8 weeks, and 12 weeks after treatment, the following items were monitored respectively: (1) Renal function: serum creatinine and blood urea nitrogen, with pre-dialysis and post-dialysis values recorded; (2) Routine blood test: hemoglobin and hematocrit; (3) Liver function: alanine aminotransferase (ALT) and aspartate aminotransferase (AST); (4) Electrolytes: serum potassium and serum sodium; (5) Adverse reactions (such as dizziness, headache, rash, edema, etc.) and corresponding treatment measures were recorded in detail.

Before treatment and 12 weeks after treatment, the following indicators were measured respectively: (1) 6-minute walk distance (6MWD): conducted in a 20-meter flat corridor in accordance with standard procedures, and the maximum walking distance was recorded; (2) Echocardiography: a GE Vivid E95 color Doppler ultrasound machine was used to estimate the pulmonary artery systolic pressure (PASP) through the tricuspid regurgitation velocity; (3) WHO functional classification: evaluated based on the patient's dyspnea and fatigue degree after daily activities.

2.3. Data analysis

All statistical analyses were performed using the SPSS 27.0 software. Continuous data were summarized as median and mean (SD), and categorical data were summarized as data counts (percentage). For the comparison of count data before and after treatment, if the difference is close to a normal distribution, the paired *t*-test was used; if the normal distribution is not satisfied, the Wilcoxon signed-rank test was adopted. The *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 10 patients were screened, and 2 patients who met the eligibility criteria were identified. Two patients were excluded: one discontinued macitentan voluntarily after 1 month, and the other lacked complete follow-up data. Patient demographics and disease characteristics are shown in **Table 1**.

Table 1. Summary of patient characteristics

Characteristics	All patients
	Total (N=10)
Female, n(%)	2 (20%)
Age, mean (SD)	49.3 (10.57)
Time of dialysis(months)	12 (10,19)
Underlying disease	

Characteristics	All patients
Glomerulonephritis	4 (40%)
Diabetic nephropathy	2 (20%)
Hypertensive nephropathy	2 (20%)
purpura nephritis	1 (10%)
Unknown	1 (10%)
Time since diagnosis, mean(SD), months	7 (4,10)
Prior PHA target therapy, n(%)	
Targeted-therapy native	9 (90%)
Targeted-therapy	1 (10%)

Patient demographics and disease characteristics are shown in **Table 1**. The mean (SD) age of the patients at baseline was 49.3 (10.57) years old, with 20% being females and the duration of dialysis and PAH diagnosis presented a skewed distribution, which was expressed as median (interquartile range), with the median dialysis duration of 12 months and the median time since PAH diagnosis of 7 months. Regarding the underlying diseases, 4 cases (40.0%) were glomerulonephritis, 2 cases (20.0%) were diabetic nephropathy, 1 case (10.0%) was purpura nephritis, and 1 case (10.0%) had an unknown underlying disease. In terms of treatment history, 9 patients (90.0%) were naive to targeted therapy for PAH, and only 1 patient (10.0%) had received prior targeted therapy. All patients received regular hemodialysis. The changes in clinical and laboratory parameters between baseline and post-treatment were compared in **Table 2**.

Table 2. Comparisons of patient characteristics before and after macitentan treatment

Measures	Baseline	First follow-up	<i>p</i> value
HB (g/L)	108.5 ± 26.142	110.1 ± 12.957	0.799
ALB (g/L)	35.470 ± 4.469	37.890 ± 3.174	0.086
ALT (U/L)	10.300 ± 3.129	13.100 ± 5.021	0.079
AST (U/L)	9.600 ± 2.757	12.6 ± 4.742	0.122
SCr (umol/L)	710.36 ± 160.634	776.810 ± 244.547	0.334
BUN (mmol/L)	15.36 (13.250, 22.323)	20.170 (14.098, 22.438)	0.799
K (mmol/L)	4.770 ± 0.868	4.660 ± 0.759	0.613
Na (mmol/L)	140.300 ± 3.466	139.500 ± 4.378	0.574
NT-proBNP (ng/L)	39018 (27674.5, 84285.5)	18987.5 (12801.5, 37959.75)	0.017
PASP	52.100 ± 5.363	39.400 ± 8.796	0.002
LVEF%	50.500 (35.00, 59.25)	53.500 (45.25, 60.75)	0.03
RVEDD (mm)	27.100 ± 2.079	26.200 ± 1.814	0.108
LVEDD (mm)	60.400 ± 11.796	59.500 ± 10.835	0.147
WHO-FC, n(%)	3 (2.75, 3)	2 (2, 3)	0.046
6MWD (mm)	234.600 ± 66.562	263.400 ± 78.062	0.035

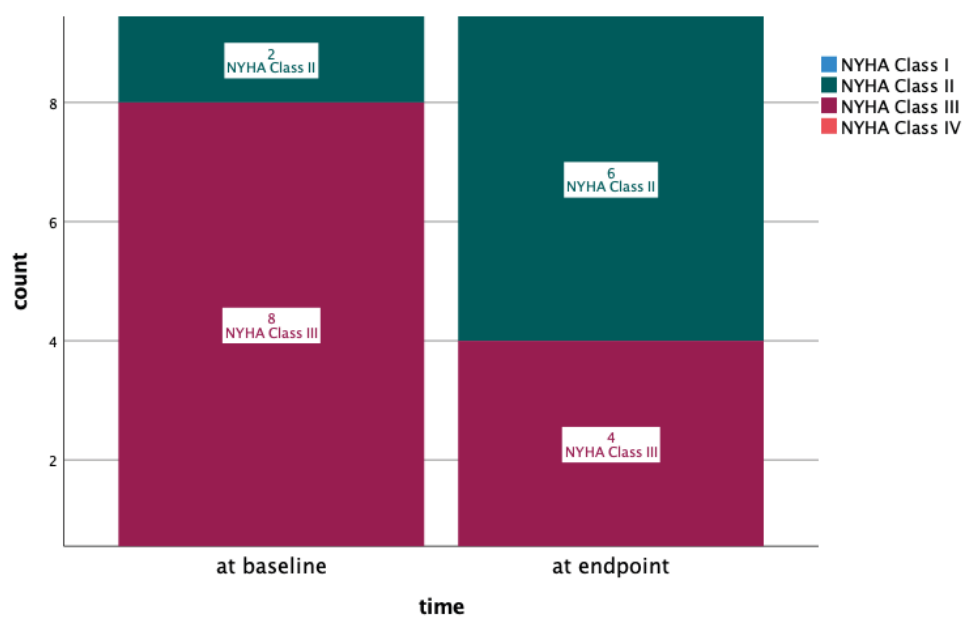


Figure 1. Change from baseline in WHO-FC at the 3-month follow-up visit.

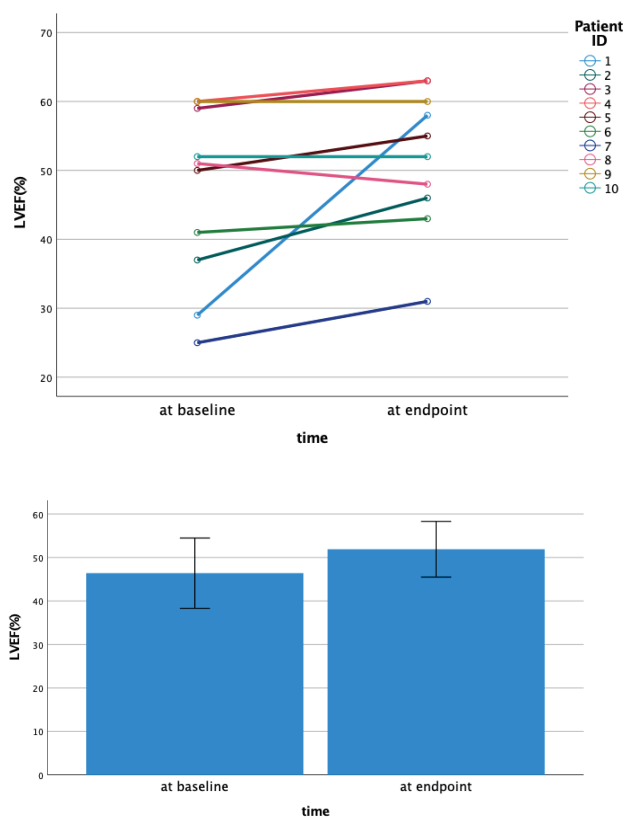


Figure 2. Change from baseline in LVEF at the 3-month follow-up visit.

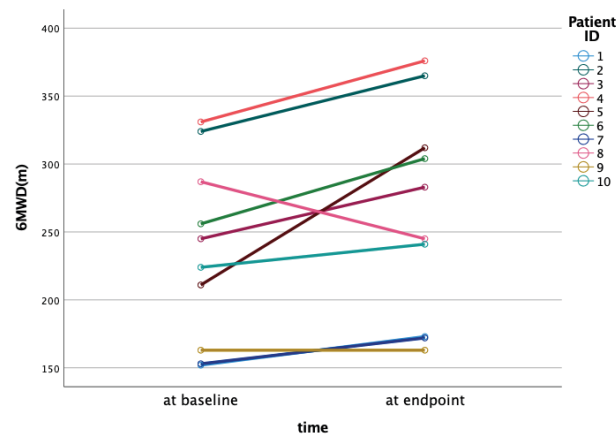


Figure 3. Change from baseline in 6MWD at the 3-month follow-up visit.

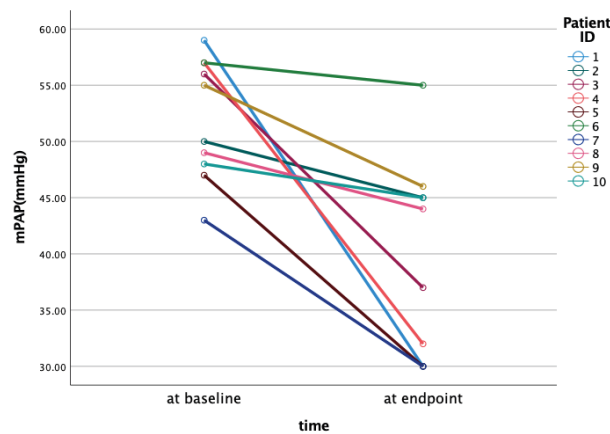
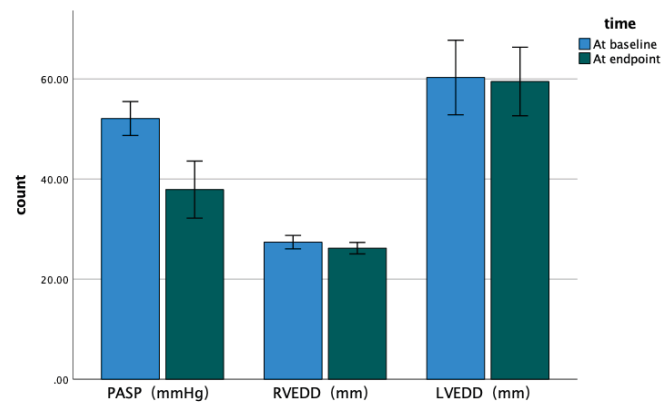


Figure 4. Change from baseline in mPAP at the 3-month follow-up visit.

3.2. Improvements in WHO-FC, 6MWD, LVEF and NT-proBNP

A total of 4 patients demonstrated improved [(n = 4), 40%] or maintained [(n = 2), 20%] WHO-FC compared to baseline (**Figure 1**). The proportion of patients with WHO-FC II changed from 20% to 60% from baseline

at the 3-month follow-up visit ($p = 0.046$). An increasing trend in the LVEF levels was also observed (**Figure 2**), with the median changing from 50.5% to 53.5% ($p = 0.03$). The mean (SD) change in 6MWD from baseline to month 3 follow-up was 28.8m ($p = 0.035$). An increase in 6MWD was observed in 7 (70%) patients, and 1 (60%) had 6MWD maintained (**Figure 3**). One patient developed a mild upper respiratory tract infection at week 8 of treatment, with a transient decrease in 6MWD from 287 m at baseline to 245 m. The 6MWD recovered to 274 m after full resolution of the infection. Significant decreases were observed in NT-proBNP levels from baseline, with median changing from 39018 ng/L to 18987.5 ng/L ($p = 0.017$).

3.3. Improvements in PASP

Mean (SD) PASP decreased from 52.1 (5.363) mmHg to 39.4 (8.796) mmHg from baseline at the 3-month follow-up visit. A decrease in PASP exceeding 10 mmHg was noted in half of the enrolled patients, with four patients achieving complete normalization of pulmonary artery pressure (**Figure 4**).

3.4. Safety

None of the 10 patients experienced death or serious adverse events during the entire treatment course. All patients-maintained ALT and AST levels within the normal range throughout the treatment period. Serum creatinine and blood urea nitrogen levels before and after dialysis showed no significant differences compared with baseline ($p > 0.05$). One patient experienced a decrease in hemoglobin from 96 g/L to 85 g/L at week 6 of treatment. Further evaluation confirmed mild blood loss caused by minor dialyzer leakage. After increasing the dose of erythropoietin from 6000 U/week to 10 000 U/week, the hemoglobin level recovered to 98 g/L at week 12 of treatment. A total of two mild adverse events occurred, accounting for an incidence of 20%. One patient developed orthostatic dizziness at week 4 of treatment, with supine blood pressure of 130/80 mmHg and upright blood pressure of 110/70 mmHg, and no syncope. After the dosage of the calcium channel blocker amlodipine was reduced from 5 mg daily to 2.5 mg daily, the dizziness completely resolved. The other patient presented with aggravated mild anemia as described above. No severe adverse reactions such as rash, edema, or hepatic injury were observed. All patients completed the 12-week treatment, and no participant discontinued the study medication.

4. Discussion

This retrospective review study showed that improvements in WHO-FC, 6MWD and pulmonary arterial hypertension for hemodialysis patients with pulmonary arterial hypertension (PAH) and New York Heart Association (NYHA) functional class II-III treated with macitentan. The therapeutic effect was consistent with the efficacy trend of previous endothelin receptor antagonists (ERA) in non-dialysis patients with pulmonary arterial hypertension^[11–13]. Since there are few previous reports on the use of macitentan in dialysis patients, the findings of this study will serve as a valuable reference for the clinical management of hemodialysis patients with PAH.

The improvements in WHO-FC, 6MWD, and NT-proBNP with macitentan at 3 months in the present study add to the growing body of evidence that supports the efficacy of macitentan in dialysis patients. In our study, we also observed numerical improvements in PASP measured by echocardiography from baseline to 3-month follow-ups. These findings build on previous evidence that macitentan improves right ventricular function and hemodynamic parameters^[14,15].

From the mechanistic perspective, pulmonary hypertension in hemodialysis patients is mainly driven by uremic toxins (indoxyl sulfate, p-cresol) that stimulate vascular endothelial cells and markedly upregulate ET-1 secretion. ET-1 triggers severe pulmonary vasoconstriction via ET-A receptors and promotes pulmonary vascular smooth muscle proliferation and collagen deposition through ET-B receptors, further accelerating pulmonary vascular remodeling ^[16,17]. As a dual endothelin receptor antagonist, macitentan blocks both ET-A and ET-B, acutely alleviating pulmonary vasoconstriction and lowering PASP, while chronically inhibiting vascular remodeling to maintain long-term efficacy ^[18,19]. In the present study, 50% of patients achieved a notable PASP reduction, and 60% improved from WHO functional class III to class II, supporting the mechanistic benefits of macitentan in hemodialysis populations. Moreover, reduced PASP decreases right ventricular afterload, optimizes right cardiac output, and improves exercise tolerance, with a mean 6MWD increase of 28.8 m. Consistent with improved cardiac functional classification, these findings indicate that macitentan can effectively ameliorate right ventricular dysfunction in hemodialysis patients.

The Adverse Drug Reactions and Serious Adverse Events observed in our study were consistent with the well-known side effects associated with approved ERAs. Regarding safety, the prescribing information for macitentan indicates that the drug is predominantly excreted via the biliary route, with only about 10% undergoing renal elimination. As hemodialysis patients, particular attention was paid to the potential risk of drug accumulation in this population. No renal function deterioration or liver injury related to drug accumulation was identified in the present study. As demonstrated in previous studies, macitentan has a favorable renal and liver safety profile ^[19,20]. In the study, only one patient experienced dizziness, which was considered associated with concurrent antihypertensive therapy, and one patient exhibited aggravated anemia, likely attributable to dialyzer leakage. Both symptoms were alleviated following symptomatic intervention, suggesting favorable short-term tolerability of macitentan among hemodialysis patients. Regular monitoring of liver function, blood pressure, and routine blood parameters during treatment can minimize the incidence of adverse reactions.

Our study has several limitations. Firstly, the small sample size, single-center setting and absence of a control group may lead to selection bias in our findings. Secondly, the observation period was only 12 weeks. Long-term efficacy and safety require further investigation with expanded samples and prolonged follow-up. Thirdly, macitentan plasma concentrations were not determined, thus limiting definitive assessment of the specific impact of hemodialysis on drug exposure.

In conclusion, macitentan is an effective therapeutic option for HD patients with WHO functional class II-III pulmonary hypertension. It improves exercise tolerance and cardiac function in the short term with a favorable safety profile. For clinical application, joint assessment by nephrology and respiratory/cardiology departments is recommended to strictly exclude contraindications, including pregnancy and hypersensitivity. Concomitant administration with CYP3A4 inhibitors such as ketoconazole should be avoided to prevent elevated drug concentrations. Long-term monitoring of blood routine, liver and renal function, and PASP is warranted, with dosing time adjusted in accordance with dialysis schedules to optimize efficacy and safety.

5. Conclusion

This study provides a rationale for the targeted use of macitentan in clinically stable hemodialysis patients with WHO functional class II–III pulmonary hypertension. Following 12 weeks of add-on macitentan 10 mg once daily alongside maintenance hemodialysis and optimized standard care, significant improvements

were observed in 6MWD, pulmonary artery systolic pressure, and WHO functional class, with favorable short-term safety and tolerability irrespective of the underlying nephropathy. Dosing schedules should be individualized to avoid concurrent dialysis to preserve therapeutic drug levels. Long-term prospective evaluation with serial assessment of calcium-phosphorus metabolism and bone mineral density is encouraged to fully characterize the long-term efficacy and safety of macitentan in this high-risk population.

Disclosure statement

The authors declare no conflict of interest.

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The Mechanisms and Research Progress of Chinese Medicine Monomers in Reversing Chemotherapy Resistance in Ovarian Cancer

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Abstract: The development of chemotherapy resistance is a critical factor leading to treatment failure and high mortality in ovarian cancer. Among current research directions, Chinese medicine monomers (CMMs) have shown great potential in reversing chemotherapy resistance due to their multi-targeting properties and low toxicity. This review summarizes the research progress of various Chinese medicine monomers in reversing chemotherapy resistance in ovarian cancer, including polyphenols (such as quercetin, curcumin, epigallocatechin-3-gallate, resveratrol, kaempferol, myricetin, and ellagic acid), alkaloids (such as berberine, piperine, sanguinarine, and capsaicin), terpenoids (such as toosendanin and triptolide), and phenylpropanoids (such as myristicin and apiole). These monomers primarily exert their effects by regulating key signaling pathways (including PI3K/AKT/mTOR, p53, and NF-κB) and by interfering with critical processes such as drug efflux, DNA damage repair, apoptosis, and autophagy, thereby enhancing the sensitivity of ovarian cancer cells to chemotherapeutic agents like cisplatin. Furthermore, this review discusses the advantages of combination strategies involving Chinese medicine monomers, as well as the current challenges.

Keywords: Chinese medicine monomers; Ovarian cancer; Chemotherapy resistance; Reversal mechanisms; Combination therapy

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1. Introduction

Ovarian cancer (OC) ranks as the 18th most common cancer worldwide, but it's the 8th leading cause of cancer death among women. In 2022 alone, an estimated 324,000 new cases and 206,000 deaths were recorded ^[1]. The standard approach, primary cytoreductive surgery followed by paclitaxel and carboplatin, works initially, yet up to 70% of women with stage III-IV high-grade disease will relapse within three years ^[2]. Why? The answer is platinum resistance. While most patients with high-grade serous ovarian cancer (HGSOC)

initially respond well, only about 20% have primary platinum-resistant disease. But for the majority who are initially sensitive, secondary resistance eventually develops after repeated recurrences. The result is a five-year survival rate that stubbornly hovers between 30% and 40% globally ^[3,4]. The sad reality is that platinum resistance catches up with nearly every OC patient over time, making it one of the strongest prognostic factors for overall survival. Clearly, we need new agents that can re-sensitize tumors to platinum-based drugs and reverse established resistance.

The roots of chemoresistance in ovarian cancer are complex and tangled. They involve multidrug resistance mechanisms, enhanced DNA damage repair, shifts in cell metabolism and oxidative stress, altered cell cycle control, the presence of cancer stem cells, immune evasion, and dysregulation of apoptosis, autophagy, and multiple signaling pathways ^[5]. Traditional Chinese Medicine (TCM) has long offered certain advantages in cancer care—fewer side effects, tailored formulas, and potential immune benefits ^[6]. But recent work shows something more direct: TCM can actually boost the sensitivity of OC cells to chemotherapy, block the cell cycle, stop proliferation, trigger apoptosis, cut off angiogenesis, reduce side effects, and improve patients' quality of life after surgery ^[7]. A growing body of evidence suggests TCM can be used alone in treating ovarian cancer or as an effective adjuvant therapy ^[8]. For example, specific Chinese medicine monomers (CMMs) have been proven to be able to modulate pathways like STAT3, PI3K/AKT, and Wnt/ β -catenin, thereby inducing apoptosis and influencing the growth and survival of ovarian cancer cells ^[7]. Other monomers, such as bufalin, can make chemotherapy work better by curbing tumor cell proliferation and nudging resistant cells toward apoptosis ^[9].

These studies provide an important theoretical foundation for the application of Chinese medicine monomers in overcoming chemotherapy resistance in ovarian cancer. However, current research on this topic is relatively fragmented and lacks systematic synthesis and analysis. This review summarizes the mechanisms and research progress of major classes of Chinese medicine monomers in reversing drug resistance in ovarian cancer, aiming to provide theoretical support for their application and future development directions in ovarian cancer treatment ^[10,11].

2. The effects and mechanisms of Chinese medicine monomers on chemotherapy resistance in ovarian cancer

2.1. Polyphenols

2.1.1. Quercetin

Quercetin, a flavonoid known for its potent antioxidant and anti-inflammatory properties, has gained attention for its multifaceted mechanisms of action against various cancers, highlighting its potential as an adjunctive therapy in cancer treatments ^[12,13]. Quercetin significantly increases the chemosensitivity of ovarian cancer cell lines, particularly OVCAR-2 and A2780P, to cisplatin and paclitaxel, suggesting its potential as an adjuvant to improve chemotherapy efficacy ^[14]. Quercetin overcomes cisplatin resistance primarily by inhibiting key proteins like MMP1, whose upregulation in resistant cells is driven by the activated MAPK signaling pathway ^[15]. Quercetin-loaded solid lipid nanoparticles enhance chemosensitization and apoptosis in ovarian cancer by downregulating the expression of multiple cisplatin resistance genes, including ABCG2, MT-2A, GST-pi, and XIAP ^[16].

2.1.2. Curcumin

Curcumin is a natural hydrophobic polyphenol compound isolated from the rhizome of *Curcuma longa* (Turmeric) ^[17]. It combats cisplatin resistance in ovarian cancer through multifaceted molecular mechanisms. Curcumin reverses cisplatin resistance in SKOV-3 ovarian cancer cells by suppressing the expression of key antioxidant enzymes and the transcription factor Nrf2, while also inhibiting the PI3K/Akt/mTOR signaling pathway, thereby confirming the role of redox-dependent regulation in the reversal of cancer cells resistance to cisplatin ^[18]. In resistant ovarian cancer cells, curcumin and its analog demethoxycurcumin downregulate glutathione S-transferase P1 (GSTP-1) and miR-133b, key players in glutathione-mediated cisplatin detoxification ^[19]. Curcumin induces demethylation of the MEG3 promoter, restoring MEG3 expression ^[20]. This upregulation of MEG3 reduces the extracellular vesicle-mediated transfer of oncogenic miR-214, which otherwise contributes to chemoresistance ^[20]. By integrating these redox-dependent, epigenetic, and signaling pathway modulations, curcumin effectively sensitizes resistant ovarian cancer cells to cisplatin.

2.1.3. Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG), a polyphenol present in green tea, offers a wide range of beneficial properties, including antioxidant, anti-inflammatory, hypoglycemic, antiviral, neuroprotective, cardioprotective, and anticancer effects ^[21–27]. It exhibits chemopreventive and therapeutic effects against ovarian cancer through multi-target mechanisms, including its dual roles as an antioxidant and pro-oxidant, epigenetic modulation via DNMT/HDAC inhibition, and suppression of key oncogenic pathways ^[28]. EGCG has been shown to effectively inhibit proliferation, mobility, and induce apoptosis in cisplatin-resistant ovarian cancer cells (OC/DDP) by downregulating the expression of S100A4 and NF-κB while upregulating p53 expression, an effect confirmed both in vitro and in vivo ^[29]. In addition, EGCG enhances cisplatin sensitivity by regulating the copper and cisplatin influx transporter CTR1; it inhibits the rapid degradation of CTR1 induced by cisplatin, increases the accumulation of cisplatin and DNA-Pt adducts, and subsequently enhances the sensitivity of ovarian cancer cells to the chemotherapeutic agent, while also exhibiting a protective effect against cisplatin-induced nephrotoxicity ^[30]. Another study found that EGCG delivers hydrogen peroxide to induce death of ovarian cancer cells, including those resistant to cisplatin, and amplifies cisplatin toxicity by three- to six-fold in various ovarian cancer cell lines by accentuating oxidative stress ^[31].

2.1.4. Resveratrol

Resveratrol is a natural polyphenol found in grapes, mulberries, peanuts, rhubarb, and several other plants, and it has antibacterial, anti-inflammatory, antioxidant, and anticancer activities ^[32,33]. This compound has a demonstrated effect against lysophosphatidic acid (LPA), a biolipid mediator that augments proliferation and metastasis of various cancer cells, including ovarian cancer invasion and metastasis ^[34]. By inhibiting the Hedgehog (Hh) pathway and restoring autophagy, resveratrol counteracts LPA-induced malignancy, supporting its inclusion in the therapy of ovarian cancer for limiting metastasis and chemoresistance ^[35]. Furthermore, resveratrol synergistically enhances the chemotherapeutic effect of cisplatin through a mechanism closely related to the significant inhibition of the PI3K/AKT/mTOR signaling pathway ^[36]. Specifically, it can prevent cisplatin-induced epithelial-to-mesenchymal transition (EMT) in ovarian cancer cells and increase cell death, demonstrating its potential as an effective adjuvant to chemotherapy ^[37]. Beyond these therapeutic mechanisms, a long-term study

demonstrated that during 26 weeks of intermittent cisplatin treatment in initially sensitive ovarian cancer A2780 cells, the continuous presence of resveratrol-maintained cisplatin chemosensitivity, whereas clinically relevant cisplatin chemoresistance developed in its absence^[38]. This suggests that resveratrol may also play a crucial role in preventing the onset of cisplatin resistance in ovarian cancer.

2.1.5. Kaempferol

Kaempferol, a dietary flavonoid widely found in fruits and vegetables, is known for its anti-inflammatory, antioxidant, and anticancer properties, and has been shown to elicit antitumor effects by controlling tumor cell cycle progression, proliferation, apoptosis, migration, and invasion, as well as by inhibiting angiogenesis^[39,40]. Mechanistically, kaempferol enhances the effect of cisplatin in ovarian cancer cells through promoting apoptosis caused by down-regulation of cMyc and inhibition of ABCC6 gene transcription^[41]. Kaempferol also increases the sensitivity of A2780 ovarian cancer cells to cisplatin by activating cytotoxic endoplasmic reticulum (ER)-mediated autophagy, evidenced by increased protein levels of GRP78, PERK, ATF6, IRE-1, LC3II, beclin 1, and caspase 4 via elevating intracellular Ca²⁺ levels, while also decreasing the protein levels of p-Akt^[42]. Furthermore, kaempferol enhances the sensitivity of cancer cells to other traditional chemotherapy drugs, such as 5-fluorouracil, making it a promising therapeutic compound for combination with current anticancer agents^[40].

2.1.6. Myricetin

Myricetin (MYR) is a flavonoid compound widely found in many natural plants, including bayberry, vegetables, fruits, nuts, and tea^[43,44]. Myricetin has demonstrated multiple biological activities, among them antidiabetic, anticancer, immunomodulatory, cardiovascular, analgesic, antihypertensive, as well as preclinical activities on Alzheimer's, Parkinson's, and Huntington's diseases^[44,45]. Myricetin is a potent natural inhibitor of CD147, a protein overexpressed in ovarian cancer that promotes malignant progression and is associated with poor prognosis. Myricetin exhibits a strong affinity for CD147 and down-regulates its protein level by facilitating proteasome-dependent degradation^[46]. Myricetin also selectively induces apoptosis in cisplatin-resistant ovarian cancer cells (OVCAR-3 and A2780/CP70) through a p53-dependent apoptotic pathway involving both Bcl-2 family-mediated intrinsic and DR5-mediated extrinsic apoptosis, without toxicity to normal ovarian cells, thereby effectively overcoming platinum-based chemoresistance^[47].

2.1.7. Ellagic acid

Ellagic acid (EA) Ellagic acid (EA) is a polyphenolic compound from the ellagitannins (ETs) family, found in various berries (such as strawberries, raspberries, blackberries, cranberries, goji berries), pomegranates, grapes, and walnuts. It has been shown to possess anti-angiogenic activity, as well as anticancer effects against various tumors and the suppression of metastasis^[48]. In the ovarian cancer A2780 cell line, ellagic acid (EA) inhibits cancer proliferation and migration by downregulating the expression of MMP2 and MMP9 both in vivo and in vitro^[49]. Furthermore, in SKOV-3 ovarian cancer cells, EA suppresses growth, migration, and invasion through the inhibition of mTORC1 and Akt, alongside the activation of AMPK-mediated cytotoxic autophagy^[50]. Regarding the mechanism of reversing ovarian cancer resistance, ellagic acid (EA) sensitizes cancer stem-like cells (CSLCs) to cisplatin treatment by enhancing the accumulation of DNA damage while simultaneously impairing their DNA repair capacity^[51]. Additionally, EA pretreatment significantly reduces

the frequency of cisplatin-induced mutations and improves intracellular drug retention, thereby suppressing the development of acquired resistance ^[51]. Another study demonstrated that ovarian cancer A2780 cells continuously treated with ellagic acid did not develop cisplatin resistance during intermittent cisplatin exposure, whereas resistance emerged in its absence, providing evidence that ellagic acid may protect against the development of cisplatin resistance in ovarian cancer ^[38].

2.2. Alkaloids

2.2.1. Berberine

Berberine, an isoquinoline quaternary alkaloid isolated from many kinds of medicinal plants such as *Coptis chinensis*, *Hydrastis canadensis*, *Berberis aristata*, *Coptis japonica*, *Phellodendron amurense* and *Phellodendron chinense Schneid*, is a nonprescription medicine traditionally used to treat diarrhea and gastroenteritis caused by bacterial and intestinal parasitic infections ^[52,53]. Accumulating evidence indicates that berberine can exert anticancer effects through multifaceted biological mechanisms across a broad spectrum of malignancies, including lung cancer, colorectal cancer, breast cancer, and ovarian cancer ^[54–57]. Berberine inhibits ovarian cancer cell growth by interfering with the expression and function of folate cycle enzymes (DHFR and TS) and upregulating the key polyamine catabolic enzyme SSAT, thereby restoring the sensitivity of drug-resistant cells to cisplatin ^[58]. Another study showed that berberine modulated the sensitivity of cisplatin in ovarian cancer A2780/DDP cells by inhibiting miR-93 expression, which in turn increased PTEN protein levels and regulated the PTEN/AKT pathway, a key signaling axis mediating miR-93-induced chemoresistance to cisplatin ^[59].

2.2.2. Piperine

Piperine is an alkaloid, a bioactive compound derived from barley (*Hordeum vulgare L.*, *Poaceae*) and black pepper ^[60]. It is gaining attention due to its ability to directly inhibit tumor growth and enhance the bioavailability of chemotherapeutic drugs ^[61]. A study demonstrated that piperine effectively increases the sensitivity of drug-resistant ovarian cancer cells to chemotherapeutic agents like paclitaxel and topotecan by concurrently targeting multiple key resistance mechanisms: upregulating protein tyrosine phosphatase receptor type K (PTPRK) expression to reduce overall protein tyrosine phosphorylation, downregulating the drug efflux pumps P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), decreasing the production of extracellular matrix proteins (COL3A1, TGFBI) involved in cell adhesion-mediated drug resistance, and inhibiting cancer cell proliferation and migration ^[62].

2.2.3. Sanguinarine

Sanguinarine, a benzophenanthridine alkaloid isolated from *Sanguinaria canadensis*, has been shown by both in vivo and in vitro experiments to exert antioxidant, anti-inflammatory, proapoptotic, and antiproliferative effects on various tumor cells ^[12,54,63]. However, its low chemical stability and poor oral bioavailability remain key issues in its use as a medicinal molecule ^[64]. Sanguinarine effectively enhances the sensitivity of cisplatin-resistant ovarian cancer A2780 cells to cisplatin and synergistically promotes apoptosis by depleting intracellular glutathione (GSH) in a dose-dependent manner, offering a potential adjuvant strategy to overcome chemoresistance in ovarian cancer ^[65]. Another study, using an integrated bioinformatics approach, identified aberrant activation of the EGFR/ErbB2 signaling pathway as a key driver of cisplatin resistance

in ovarian cancer and discovered that the natural product sanguinarine effectively targets this resistance network, significantly suppressing the growth of both sensitive and resistant ovarian cancer cells in vitro and inhibiting xenograft tumors in vivo ^[5].

2.2.4. Capsaicin

Capsaicin, a bioactive alkaloid derived from chili peppers, produces antioxidative, antitumor, antiulcer and analgesic effects and has demonstrated potential as a treatment for cardiovascular, gastrointestinal, oncological and dermatological conditions ^[66]. In ovarian cancer, capsaicin exhibits growth-suppressive activity and sensitizes cancer cells to the pro-apoptotic effects of chemotherapy ^[67]. Mechanistically, capsaicin modulates key regulators of chemoresistance, including the inhibition of P-glycoprotein (P-gp), the nuclear factor-kappa B (NF-κB) pathway, and the signal transducer and activator of transcription 3 (STAT3) pathway, thereby contributing to the reversal of multidrug resistance (MDR) ^[68]. In human ovarian cancer cell lines, including the cisplatin-resistant A2780(cisR) model, combinations of capsaicin with a novel platinum agent demonstrated synergistic activity, indicating its potential to enhance the efficacy of existing chemotherapeutics ^[69].

2.3. Terpenoid

2.3.1. Toosendanin

Toosendanin (TSN) is a tetracyclic triterpenoid derived from *Melia toosendan* and *M. azedarach* ^[70]. Modern studies have shown that TSN has strong anti-tumor, anti-botulinum, anti-viral and anti-parasitic potential ^[71]. Concerning the reversal of drug resistance, TSN can reduce DDP resistance in OC through regulating the miR-195/ERK/β-catenin pathway, highlighting the potential of TSN as an effective agent for overcoming clinical DDP resistance in OC both in vitro and in vivo ^[72].

2.3.2. Triptolide

Triptolide (TPL) is a biologically active diterpenoid triepoxide extracted from the traditional Chinese medicine *Tripterygium wilfordii*. It has presented potent pharmacological activity and anti-cancer, anti-tumor, anti-obesity and anti-diabetes effects ^[73,74]. TPL antagonizes cisplatin resistance in human ovarian cancer cell line A2780/CP70 via hsa-mir-6751 ^[75]. Triptolide triggers lethal autophagy in cisplatin-resistant SKOV3/DDP ovarian cancer cells through the ROS-mediated inhibition of the JAK2/STAT3 signaling pathway ^[76]. TPL negatively regulated the NF-κB pathway through mitochondria-derived ROS accumulation, promoting the apoptosis of the SKOV3PT cells, increasing the sensitivity of cells to chemotherapy-dependent apoptosis and reversing drug resistance in ovarian cancer ^[77].

2.4. Phenylpropanoids

2.4.1. Myristicin

Myristicin is an active natural compound from the alkylbenzene family, mainly found in nutmeg (*Myristica fragrans*), also present in parsley (*Petroselinum crispum*), black pepper (*Piper nigrum*), carrots (Umbelliferae family) and plants of the Apiaceae family ^[78]. Previous research showed that myristicin possesses anti-inflammatory, antimicrobial, anti-proliferative and anticancer properties, including hepatic carcinoma, gastric cancer and breast cancer ^[79–82]. Myristicin itself lacks direct cytotoxicity against multidrug-resistant ovarian cancer cells, but it significantly enhances the cytotoxic effects of chemotherapeutic agents like cisplatin

and docetaxel by binding to and blocking the efflux function of P-glycoprotein (P-gp), thereby reversing multidrug resistance in ovarian cancer ^[78].

2.4.2. Apiole

Apiole is a phenylpropanoid found in parsley (Apiaceae), but also in the fruits of *Petroselinum crisp*, the seeds of *Enterolobium contortisiliquum*, and caraway (*Carum carvi L*) ^[83]. Apiole exhibits various pharmacological activities, including antioxidant, antibacterial, and anti-inflammatory effects, and has been identified as a potent calcium channel inhibitor ^[83,84]. In the context of ovarian cancer, Apiole reverses multidrug resistance in ovarian cancer cells by binding to the active site of P-glycoprotein (P-gp) (involving residues such as Phe728 and Tyr303), as confirmed by molecular docking, thereby inhibiting the efflux of chemotherapeutic drugs like doxorubicin and vincristine and synergistically enhancing their antiproliferative effects ^[85]. This suggests that apiole acts as an effective P-gp inhibitor and is a promising adjuvant agent to overcome multidrug resistance in ovarian cancer.

Table 1. Chinese medicine monomers reversing chemotherapy resistance in ovarian cancer

Monomer Class	Monomer Name	Mechanism of Action
Polyphenols	Quercetin	Inhibits MMP1; downregulates ABCG2, MT-2A, GST-pi, XIAP
	Curcumin	Inhibits PI3K/Akt/mTOR and Nrf2; modulates MEG3/miR-214 axis; downregulates GSTP-1 and miR-133b.
	Epigallocatechin-3-gallate (EGCG)	Regulates S100A4/NF-κB/p53 axis; upregulates CTR1; enhances oxidative stress
	Resveratrol	Inhibits Hedgehog/PI3K/AKT pathways; blocks EMT; prevents resistance development
	Kaempferol	Downregulates cMyc/ABCC6; activates ER stress-mediated autophagy
	Myricetin	Induces p53-dependent apoptosis; inhibits CD147
	Ellagic acid	Enhances DNA damage and impairs repair; improves drug retention
Alkaloids	Berberine	Interferes with folate enzymes (DHFR, TS); upregulates SSAT; regulates miR-93/PTEN/AKT axis.
	Piperine	Upregulates PTPRK; downregulates P-gp/BCRP; reduces ECM proteins (COL3A1, TGFBI).
	Sanguinarine	Depletes glutathione (GSH); targets EGFR/ErbB2 network.
	Capsaicin	Inhibits P-gp, NF-κB, and STAT3 pathways.
Terpenoids	Toosendanin	Regulates miR-195/ERK/β-catenin pathway.
	Triptolide	Modulates hsa-mir-6751/HK2, ROS/JAK2/STAT3, and ROS/NF-κB pathways
Phenylpropanoids	Myristicin	Blocks P-gp efflux function.
	Apiole	Binds to P-gp active site, inhibiting drug efflux.

Note: EGCG, epigallocatechin-3-gallate; EMT, epithelial-mesenchymal transition; ER, endoplasmic reticulum; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; ECM, extracellular matrix; GSH, glutathione; ROS, reactive oxygen species. Numbers in square brackets are reference citations.

3. Benefits of the combination of TCM monomers

Several studies have investigated the efficacy of mixtures combining two or three different Chinese

medicine monomers. The combination of curcumin and resveratrol reverses chemoresistance in cisplatin-resistant epithelial ovarian cancer cells by synergistically inhibiting the PI3K/AKT/mTOR pathway ^[36]. When the novel monofunctional platinum agent LH6 was combined with curcumin and quercetin, it resulted in significantly enhanced cytotoxic effects against human ovarian cancer cells, confirming a synergistic interaction ^[86]. In a co-culture model of ovarian cancer and mesenchymal stem cells, the combination of fisetin and quercetin reversed microenvironment-induced chemoresistance by restoring ERK1/2 phosphorylation, thereby re-sensitizing cells to platinum drugs ^[87]. The combination of epigallocatechin gallate (EGCG) and sulforaphane (SFN) significantly enhances the efficacy of cisplatin against both sensitive and resistant ovarian cancer cells by potentiating G2/M phase arrest and upregulating p21 expression, offering a novel approach to overcoming chemotherapy resistance ^[88]. Another study found the sequenced combination of curcumin and EGCG added 4 hours after administering cisplatin produced the strongest synergistic inhibitive effect in both sensitive and resistant ovarian cancer cells by increasing cellular platinum accumulation and platinum-DNA binding levels ^[89]. The combination of curcumin and triptolide could synergistically inhibit ovarian cancer cell growth and induce apoptosis, which is accompanied by HSP27 and HSP70, indicating that HSP27 and HSP70 play an important role in this synergic effect ^[90].

Overall, polyphenols, alkaloids, terpenoids, and phenylpropanoids, as natural compounds derived from traditional Chinese medicine, exhibit potent preventive and therapeutic effects against ovarian cancer through pleiotropic pathways and mechanisms, including the reversal of chemotherapy resistance.

4. Challenges, limitations, and prospects

Although these traditional Chinese medicine monomers have obvious anti-cancer potential, they generally have poor bioavailability, which limits their clinical application. For example, phenols and terpenoids have poor solubility and low permeability. Alkaloids have efflux activity and are metabolized quickly. Phenylpropanoids are toxic ^[91–97]. These characteristics will cause the drug to fail to reach an effective therapeutic concentration in tumor tissues.

In response to these limitations, drug delivery systems based on nanotechnology have been developed. It includes nanoparticles, liposomes, micelles, dendritic polymers, nanocapsules and nanostructured lipid carriers. After encapsulating drugs with them, the pharmacokinetic characteristics of traditional Chinese medicine monomers can be improved, and the controlled release of drugs and tumor-specific accumulation can be achieved ^[98–100]. For example, in a study, lipid-chitosan hybrid nanoparticles were used to co-deliver curcumin and cisplatin, which significantly enhanced the cytotoxicity to ovarian cancer cells by increasing chemical sensitization and achieving controlled drug release ^[101]. Another study showed that quercetin-loaded solid lipid nanoparticles effectively reversed the resistance of ovarian cancer cells to cisplatin by down-regulating key drug resistance genes (such as ABCG2, MT-2A, GST-pi and XIAP), while inhibiting cell migration and promoting cell apoptosis, thereby improving the chemotherapy effect ^[16].

Another solution is to develop and synthesize derivatives or analogues by chemical modification. For example, the combination of hydrophilic groups or the design of prodrugs is another effective strategy to improve the solubility and metabolic stability of traditional Chinese medicine monomers, thereby enhancing their bioavailability ^[102,103]. For example, the curcumin derivative NL01 not only showed significantly stronger anti-tumor efficacy than its parent compound but also induced ferroptosis in ovarian cancer cells by

down-regulating the HCAR1 / MCT1 signaling pathway, thereby overcoming therapeutic limitations^[104].

In terms of research design, there are still several key limitations in the current literature. One of the most significant is the lack of clinical data. At present, most studies are still limited to animal models and in vitro cell experiments, and there is a lack of well-designed clinical trials and real-world studies to verify the specific efficacy and mechanism of traditional Chinese medicine monomers in the patient population. In addition, the existing research mainly focuses on a single monomer. There are obvious research gaps in exploring the synergistic effect of combined traditional Chinese medicine monomers on reversing chemotherapy resistance or enhancing chemotherapy sensitivity of ovarian cancer. It is worth pondering whether the combination of different Chinese medicine monomers or their active ingredients to target the same pathway can produce enhanced therapeutic effects, which is also a problem that needs further study.

5. Conclusion

The emergence of drug resistance remains a major challenge in the treatment of ovarian cancer. Traditional Chinese medicine monomers have shown the potential to reverse the drug resistance of ovarian cancer through a variety of ways and mechanisms, which is crucial for future treatment strategies. Traditional Chinese medicine monomers such as quercetin, curcumin, piperine, and triptolide have shown great potential to reverse ovarian cancer chemotherapy resistance through multiple key molecular pathways (including PI3K / AKT / mTOR, p53, and NF-κB). However, their clinical application is limited by challenges such as poor bioavailability and insufficient clinical trials. In order to solve these limitations, nanotechnology-based drug delivery systems, synthetic derivatives and combined treatment strategies have been developed to improve pharmacokinetic characteristics and therapeutic effects. Future research should focus on the study of joint strategies and conduct larger-scale, well-designed clinical trials. The goal is to verify the reversal effect of traditional Chinese medicine monomers on the drug resistance of ovarian cancer and to develop more effective drug regimens.

Disclosure statement

The authors declare no conflict of interest.

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Spatial Mapping Reveals an Immunosuppressive Niche Linking CD8A⁺ T-cell Exhaustion and Breast Cancer Stem Cells in HER2-Positive Breast Cancer

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Abstract: *Objective:* This study aimed to characterize the spatial distribution of CD8A⁺PD-1⁺TIGIT⁺ exhausted T cells in HER2-positive breast cancer and to investigate their spatial relationship with CD44⁺CD24⁻ breast cancer stem cells. *Method:* Formalin-fixed paraffin-embedded HER2-positive breast cancer specimens were analyzed using multiplex immunofluorescence, pathological region annotation, and spatial proteomic analysis. Regions of interest were annotated based on histological features, including invasive carcinoma-associated areas, adjacent stroma, peritumoral fibrous tissue, and adipose tissue. CD8A⁺PD-1⁺TIGIT⁺ T cells were identified at the single-cell level, and their regional distribution was quantitatively compared. Spatial mapping, distance-based analysis, and interaction network analysis were performed to evaluate the spatial association between exhausted T cells and CD44⁺CD24⁻ BCSCs. Pairwise comparisons were conducted using two-tailed Welch's t-test. *Result:* CD8A⁺PD-1⁺TIGIT⁺ T cells were preferentially enriched in invasive carcinoma-associated regions and adjacent stromal areas, with significantly lower abundance in peritumoral fibrous and adipose tissues. Within the CD8A⁺ T-cell compartment, the proportion of triple-positive exhausted T cells was highest in invasive lesions and decreased toward peripheral non-invasive regions. Spatial analyses showed that CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ BCSCs were co-enriched in invasive carcinoma, tumor-stromal interface zones, and lymphoid tissue-associated microregions. Distance-based heatmaps and interaction network analyses further confirmed recurrent spatial proximity between these two cell populations. *Conclusion:* CD8A⁺PD-1⁺TIGIT⁺ exhausted T cells are spatially enriched in invasive pathological microregions of HER2-positive breast cancer and show close spatial association with CD44⁺CD24⁻ BCSCs. The observed spatial overlap between tumor stemness and T-cell exhaustion defines a localized suppressive niche. This interplay likely underpins the biological mechanisms through which HER2-positive breast cancer evades immune surveillance and develops resistance to therapy.

Keywords: HER2-positive breast cancer; T-cell exhaustion; Breast cancer stem cells; Spatial proteomics;

1. Introduction

Characterized by HER2 protein overexpression or gene amplification, the HER2-positive subtype represents approximately 15–20% of all breast cancer diagnoses. While the clinical landscape has been transformed by HER2-targeted therapies, ranging from monoclonal antibodies to antibody-drug conjugates and tyrosine kinase inhibitors, treatment success is often hindered by both primary and secondary resistance^[1]. Increasingly, the tumor immune microenvironment is recognized as a pivotal factor governing these therapeutic outcomes.

Unlike many other breast cancer variants, the HER2-positive subtype is often characterized by a more robust immune landscape. The presence of tumor-infiltrating lymphocytes, particularly CD8⁺ T cells, frequently aligns with better clinical outcomes and a stronger response to anti-HER2 treatments^[2]. This synergy is partly explained by the fact that HER2 antibodies do not just block signaling; they also engage the immune system via mechanisms such as antibody-dependent cellular cytotoxicity (ADCC). This interplay establishes a clear biological foundation for pairing HER2 inhibitors with immunotherapies^[3,4].

Antitumor immunity is largely orchestrated by immune checkpoint pathways, which maintain the balance between activation and suppression. PD-1, for instance, drives T-cell impairment and exhaustion when it interacts with its ligands, PD-L1 or PD-L2, on activated T cells. Similarly, TIGIT functions as a critical inhibitory marker on exhausted CD8⁺ T cells, regulatory T cells, and NK cells^[5]. It curtails effector responses by outcompeting CD226 for binding to shared ligands CD155 and CD112, thereby facilitating immune evasion^[6]. Because PD-1 and TIGIT are frequently co-expressed during T-cell dysfunction, targeting both pathways simultaneously may offer a more potent strategy for revitalizing immune surveillance than inhibiting either alone^[7].

Standard analytical tools often fail to capture the full immune complexity of HER2-positive tumors. While H&E staining defines histological structure, it cannot assess the functional status or checkpoint co-expression of infiltrating lymphocytes. Conversely, bulk transcriptomic and proteomic profiling offer high molecular resolution but sacrifice spatial context. This limitation is critical in breast cancer, where pronounced regional heterogeneity means that immune landscapes can vary significantly within a single lesion^[8]. Consequently, resolving these spatial dynamics requires advanced approaches that integrate molecular depth with anatomical integrity.

Spatial proteomic platforms, such as multiplex immunofluorescence, bridge the gap between high-dimensional molecular profiling and tissue morphology by detecting multiple markers in situ^[9]. These technologies facilitate the precise quantification of immune subsets, checkpoint densities, and exhaustion phenotypes within pathologically defined zones. By mapping these localized immunosuppressive niches, these platforms provide a critical framework for identifying and selecting rational therapeutic targets in HER2-positive breast cancer^[10].

To resolve the spatial architecture of T-cell exhaustion in HER2-positive breast cancer, we integrated multiplex immunofluorescence with pathological annotation and spatial interaction analysis. We characterized the distribution of CD8A⁺PD-1⁺TIGIT⁺ exhausted T cells across distinct compartments,

including invasive carcinoma, stroma, and peritumoral fibrous or adipose tissues, while evaluating their proximity to CD44⁺CD24⁻ breast cancer stem cells (BCSCs). By combining regional quantification with spatial network analysis, we sought to delineate localized immunosuppressive niches and provide evidence for a spatial link between T-cell exhaustion and the BCSC-enriched microenvironment.

2. Materials and methods

2.1. Clinical specimens

FFPE specimens from HER2-positive breast cancer patients were utilized for both pathological assessment and spatial proteomic analysis. Corresponding clinicopathological data (e.g., age, tumor size, metastasis, grade, stage, and molecular markers such as ER/PR/HER2/Ki-67) were extracted from hospital pathology reports. This study received approval from the local Ethics Committee and adhered to the ethical principles for human subject research. Written informed consent was secured from all patients prior to enrollment.

2.2. Pathology methods

All hematoxylin and eosin-stained and immunohistochemically stained slides were reviewed by two experienced pathologists. Histological type, tumor grade, invasive tumor components, lymph node status, and pathological stage were evaluated according to standard breast cancer pathological criteria. Tumor histological grade was assessed using the Elston and Ellis scoring system. Immunohistochemical staining for ER, PR, HER2, and Ki-67 was performed on 4-μm formalin-fixed paraffin-embedded tissue sections according to routine clinical protocols. ER and PR positivity was defined as nuclear staining in $\geq 1\%$ of tumor cells. HER2 status was scored as 0, 1⁺, 2⁺, or 3⁺ by immunohistochemistry, and fluorescence in situ hybridization was performed for cases with equivocal HER2 expression. HER2 positivity was defined as IHC 3⁺ or HER2 gene amplification by fluorescence in situ hybridization. The Ki-67 index was calculated as the percentage of positively stained tumor nuclei in invasive tumor areas. All pathological and immunohistochemical features were assessed based on the invasive tumor components.

For spatial proteomic analysis, representative tumor regions were selected on the basis of corresponding hematoxylin and eosin-stained sections. Regions of interest were annotated by pathologists to include invasive tumor areas and relevant tumor microenvironment regions, while areas with necrosis, tissue folding, poor fixation, or obvious artifacts were excluded.

2.3. Multiplex immunohistochemistry

Multiplex immunohistochemistry/immunofluorescence staining was performed on formalin-fixed paraffin-embedded breast cancer tissue sections. After deparaffinization, rehydration, antigen retrieval, and blocking, sections were sequentially incubated with primary antibodies against immune and functional markers, including CD8, PD-1, TIGIT, and other markers in the panel. Each staining cycle was followed by horseradish peroxidase-conjugated secondary antibody incubation, tyramide signal amplification, and antibody stripping before the next marker was applied. After all staining cycles, nuclei were counterstained with DAPI, and slides were mounted with antifade medium.

Multispectral images were acquired using a fluorescence imaging system. Single-stained controls were used to establish the spectral library and perform spectral unmixing. The unmixed images were subsequently used for tissue segmentation, cell segmentation, phenotyping, and spatial analysis. CD8-positive T cells co-

expressing PD-1 and/or TIGIT were identified at the single-cell level and analyzed within pathologically annotated regions of interest.

2.4. Spatial proteomics

The general process of spatial proteomics is shown in **Figure 1**. Regions of interest were first found in Phenochart and then analysed in inForm to conduct spectral unmixing, tissue segmentation, cell segmentation and phenotype assignment. Export images of the components and cell-level outputs, such as marker intensities, tissue classifications, cell coordinates and phenotype annotations, for further spatial analysis.

Unmixed images were then imported into QuPath for image stitching and data organisation. Manually annotated tissue compartments and performed cell segmentation using StarDist. Based on the pattern of marker expression and morphology, trained object classifiers were used to assign cell phenotypes. QuPath's spatial analysis functions were then used to measure and plot the distance between selected cell populations, such as CD8⁺ T cells and PD-1- or TIGIT-expressing subsets.

QuPath was used to analyse cell-level data in CytoMAP further for neighbourhood studies. Neighborhoods were defined as 50-µm raster-scanned windows, and the relative abundance of each cell phenotype was quantified within these neighborhoods. Some areas have been divided according to the type of cells. Then, neighborhood composition heatmaps and interaction networks were used to study the spatial distribution and relationships among immune cells in regional associations of tumours to build models of the tumour microenvironment.

2.5. Statistics

Statistical analyses were performed to compare the proportion of CD8A⁺PD-1⁺TIGIT⁺ exhausted T cells among different regions of interest. Data are presented as mean ± standard deviation. Pairwise comparisons between ROI groups were conducted using a two-tailed Welch's t-test, which does not assume equal variance between groups. Statistical significance is indicated as **P* < 0.05.

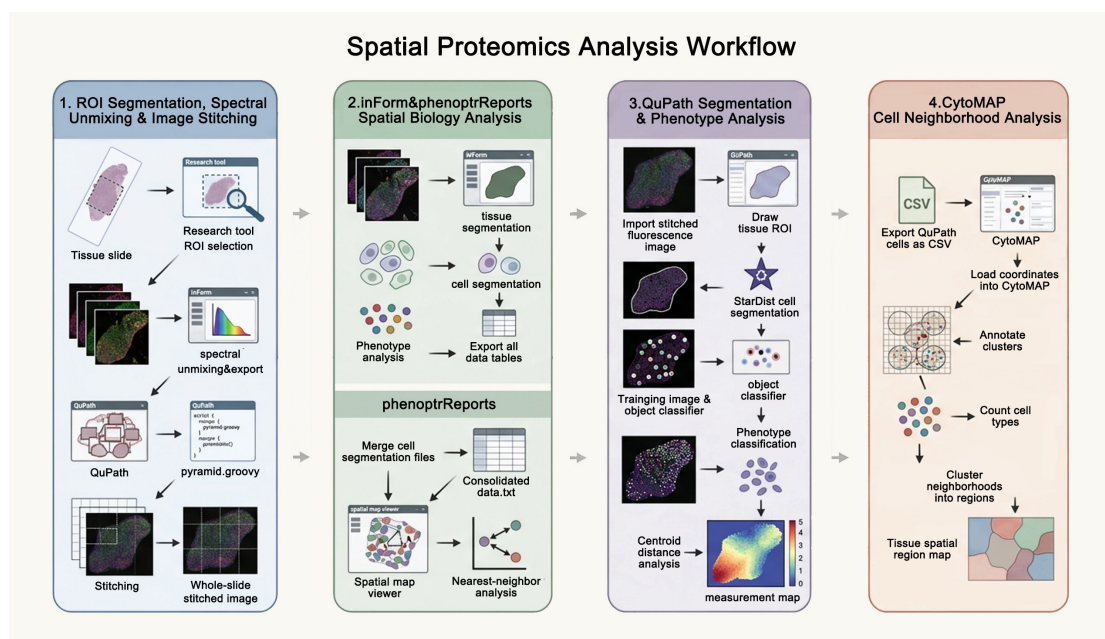


Figure 1. The overall workflow of spatial proteomic analysis.

3. Results

3.1. Spatial enrichment of CD8A⁺PD-1⁺TIGIT⁺ T cells in invasive pathological microregions of HER2-positive breast cancer

To assess whether T-cell exhaustion varies across regions of the HER2-positive breast cancer tumor microenvironment, we first focused on the CD8A⁺PD-1⁺TIGIT⁺ T-cell subset. CD8A⁺ T cells are major mediators of cytotoxic antitumor immunity, whereas PD-1 and TIGIT are inhibitory receptors closely associated with exhaustion-related immune suppression. Their co-expression on CD8A⁺ T cells may therefore indicate a more advanced exhausted phenotype. On this basis, we analyzed the spatial distribution of this cell population across pathologically distinct microregions.

A representative tumor section from a patient with HER2-positive breast cancer was selected for analysis. Based on histological architecture and pathological features, the section was divided into three regions of interest (ROIs). ROI1 mainly contained invasive carcinoma intermingled with stromal components. ROI2 included invasive carcinoma and stroma, with multifocal lymphocytic infiltration and areas of lymphoid tissue hyperplasia. ROI3 was composed primarily of peritumoral fibrous and adipose tissue. Multiplex immunofluorescence staining for CD8A, PD-1, and TIGIT was then performed to detect CD8A⁺PD-1⁺TIGIT⁺ triple-positive cells, together with other CD8A⁺ T-cell phenotypes (**Figures 2A–C**).

In this representative section, CD8A⁺PD-1⁺TIGIT⁺ T cells accounted for the largest proportion in ROI1, decreased in ROI2, and were least frequent in ROI3 (**Figure 2D**). Further phenotypic stratification of the CD8A⁺ T-cell compartment showed that triple-positive cells represented a relatively enriched subset in ROI1, whereas CD8A⁺PD-1[−]TIGIT[−] cells were more prominent in ROI3 (**Figure 2E**). These findings suggest that regions associated with invasive carcinoma may not only attract CD8A⁺ T cells but also preferentially contain cells co-expressing PD-1 and TIGIT, consistent with a locally enhanced exhausted phenotype.

The region-specific pattern observed in the representative case was further examined in pathological sections from six additional patients with HER2-positive breast cancer. Quantitatively, CD8A⁺PD-1⁺TIGIT⁺ T cells were significantly more concentrated in ROI1, and then decreased step-wise in ROI2 and ROI3 (**Figure 2F**). Analysis of the composition of CD8A⁺ T cells showed a similar trend: the fraction of triple-positive cells decreased from the invasive carcinoma-associated region to the peritumoral tissue, and CD8A⁺PD-1[−]TIGIT[−] cells showed a relative increase in the opposite direction (**Figure 2G**). Based on the above results, CD8A⁺PD-1⁺TIGIT⁺ T cells are not uniformly distributed in the tumour microenvironment. Instead, they are more likely to be located in invasive carcinoma-associated microregions, and thus support the existence of a spatially organised pattern of T-cell exhaustion in HER2-positive breast cancer.

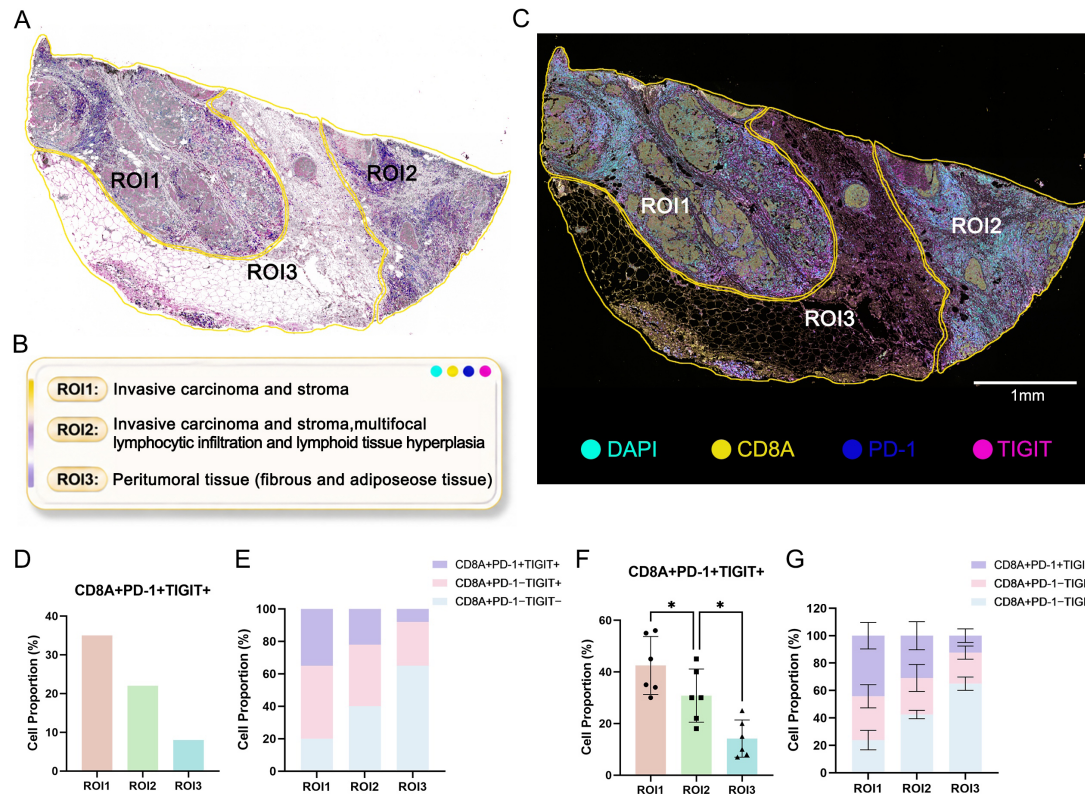


Figure 2. Spatial Enrichment of CD8A⁺PD-1⁺TIGIT⁺ T Cells in Invasive Pathological Microregions of HER2-Positive Breast Cancer. (A) Panoramic view of a representative pathological section from a HER2-positive breast cancer patient, divided into three regions of interest (ROIs) according to histological architecture. (B) Summary of pathological features of the three ROIs. (C) Multiplex immunofluorescence image of the representative section showing DAPI, CD8A, PD-1, and TIGIT staining, together with the spatial locations of the three ROIs. Scale bar, 1 mm. (D) Proportion of CD8A⁺PD-1⁺TIGIT⁺ cells in the three ROIs of the representative section. (E) Phenotypic composition of CD8A⁺ T-cell subsets in the three ROIs of the representative section, including CD8A⁺PD-1⁺TIGIT⁺, CD8A⁺PD-1⁻TIGIT⁺, and CD8A⁺PD-1⁻TIGIT⁻ cells. (F) Quantitative analysis of the proportion of CD8A⁺PD-1⁺TIGIT⁺ cells across the three ROIs in pathological sections from six HER2-positive breast cancer patients. (G) Compositional analysis of CD8A⁺ T-cell phenotypes across the three ROIs in six HER2-positive breast cancer patients. Data are presented as mean \pm SEM. * $P < 0.05$.

3.2. CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ breast cancer stem cells are spatially co-enriched in invasive lesions

After observing the preferential accumulation of CD8A⁺PD-1⁺TIGIT⁺ T cells in invasive carcinoma-associated regions, we next evaluated whether this exhausted T-cell subset was spatially associated with specific tumor cell niches. The CD44⁺CD24⁻ phenotype is widely used to identify breast cancer stem-like cells and has been associated with tumor invasion, recurrence, treatment resistance, and immune escape^[11,12]. We therefore analyzed the spatial relationship between CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ BCSCs in the same HER2-positive breast cancer section, to determine whether cancer stem-like cell-enriched areas were accompanied by local enrichment of exhausted T cells.

Panoramic spatial mapping showed that neither CD8A⁺PD-1⁺TIGIT⁺ T cells nor CD44⁺CD24⁻ BCSCs were uniformly distributed in the tissue section. Instead, both groups were mainly clustered in areas of

invasive carcinoma and the adjacent stroma (**Figure 3A**). On the other hand, the adipose- and fibre-poor area far from the tumour parenchyma was relatively sparse. A distance-based spatial heatmap also shows that the intercellular distance is shorter between the two groups, specifically in invasive carcinoma and the surrounding stromal area, indicating that these pathological micro-regions are closer in space (**Figure 3B**). The above distribution indicates that the regional accumulation of CD8A⁺PD-1⁺TIGIT⁺ T cells is unlikely to be due to general inflammation; instead, it may be linked to a specific microenvironment in that area with certain types of cancer cells.

High-magnification observation of individual areas also confirmed the above. In ROI1, CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ BCSCs were mainly co-distributed in the invasive carcinoma lesions and adjacent stromal areas, with a prominent accumulation at the interface among tumor parenchyma, lymphocyte-enriched regions, and fibrous stroma (**Figures 3C, D, F, G**). Although the tissue architecture in ROI2 was more heterogeneous, both populations were still mainly restricted to invasive carcinoma and marginal tumor regions, and they were in close spatial proximity at the tumor-stroma interface (**Figures 3E, H, I, L**). In several other representative subregions, the two cell populations were similarly enriched near invasive carcinoma lesions and adjacent stroma, and their abundance was significantly reduced in adipose tissue and fibrous areas away from the tumor parenchyma (**Figures 3J, K, M, N**).

Based on the above results, it can be concluded that CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ BCSCs are spatially co-enriched in HER2-positive breast cancer, primarily located in invasive carcinoma, tumor-stromal interface zones, and lymphoid tissue-associated microregions. Therefore, the spatial arrangement may create a niche for cancer stem-cell-enriched cells that induces local immune exhaustion, and together the two will constitute an immunosuppressive pathological microenvironment.

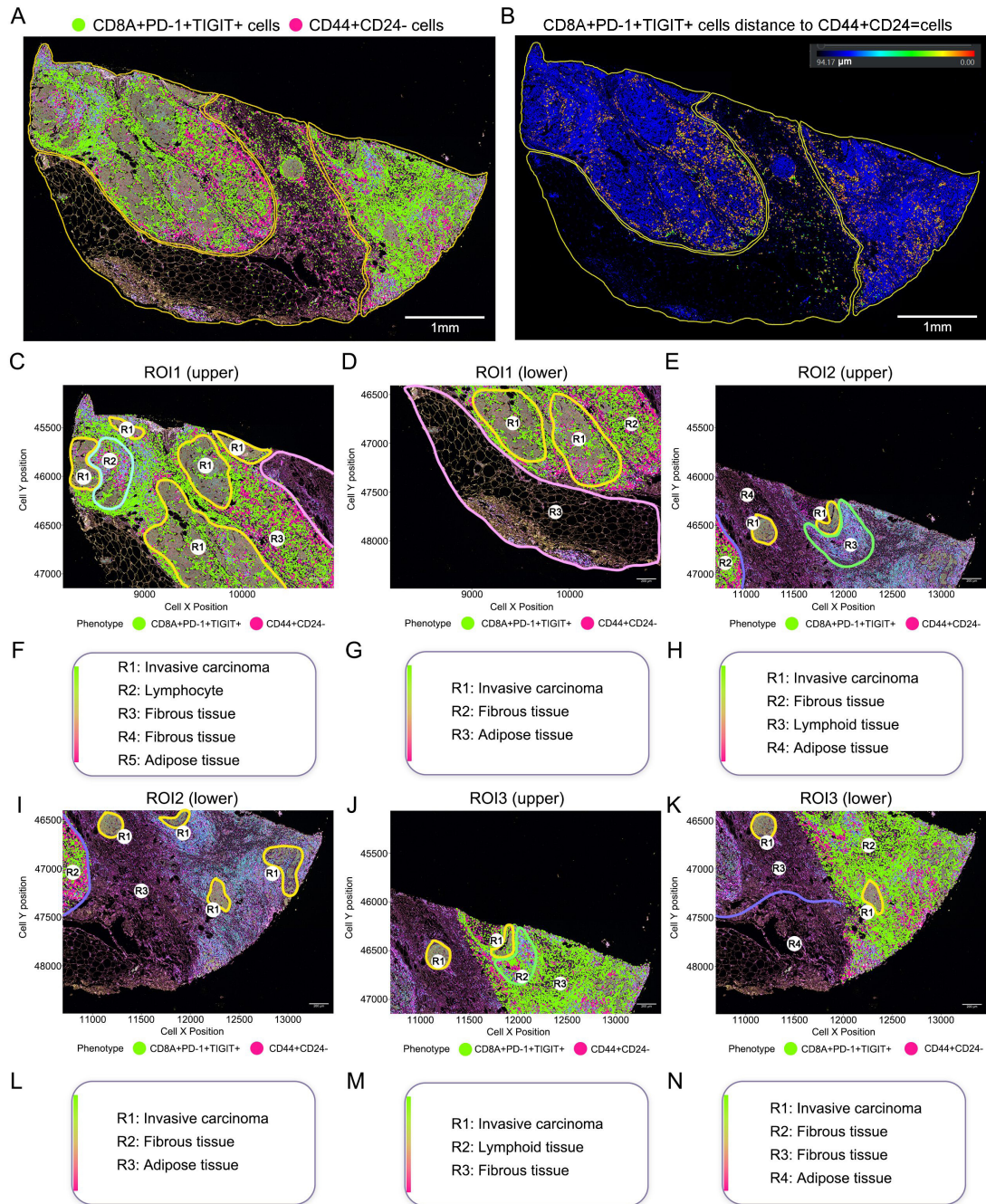


Figure 3. CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ breast cancer stem cells are spatially co-enriched in invasive lesions. (A) Panoramic spatial distribution of CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ BCSCs in a representative HER2-positive breast cancer section. Fluorescent green indicates CD8A⁺PD-1⁺TIGIT⁺ T cells, and magenta indicates CD44⁺CD24⁻ BCSCs. Scale bar, 1 mm. (B) Distance-based spatial heatmap showing the proximity between the two cell populations. Warmer colors indicate shorter distances, whereas cooler colors indicate greater distances. Scale bar, 1 mm. (C–E, I–K) Magnified representative regions from ROI1 and ROI2 showing the spatial distribution of the two cell populations across invasive carcinoma, lymphoid/lymphocyte-enriched areas, fibrous tissue, and adipose tissue. Scale bars, 200 μ m. (F–H, L–N) Corresponding pathological annotations for the magnified regions shown in C–E and I–K.

3.3. Spatial interaction network analysis further confirms the proximity between CD8A⁺PD-1⁺TIGIT⁺ T Cells and CD44⁺CD24⁻ breast cancer stem cells

Building on the observation that CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ BCSCs are co-enriched, we wanted to know if this co-distribution represented a close, measurable association. Therefore, a spatial interaction network and matrix analysis were conducted on multiple ROIs. The study models the distances between cells as network edges and matrix-based affinity scores to systematically compare the spatial relationships among exhausted T cells, BCSCs, and other cellular components in the microenvironment.

Both subregions in ROI1 showed a clear spatial proximity of the two cell types (**Figures 4A–B**). Interaction matrices have quantified these associations and revealed positive interaction signals and self-aggregation in the exhausted CD8A⁺ T-cell compartment (**Figures 4D, E**). Given that the interaction signals are stronger in the lower subregion, it is estimated that the spatial interface of exhausted T cells and BCSCs may be context-dependent and influenced by heterogeneous histological structures within the tumour.

Parallel spatial patterns were observed in ROI2, and both subregions were close to exhausted T cells and BCSCs (**Figures 4C, G**). Quantitative analysis of interaction matrices also shows that these areas are not spatially segregated in terms of tissue structure (**Figures 4F, J**). The reappearance of this pattern, especially the enhanced signal in the lower subregion, indicates that the association between CD8A⁺PD-1⁺TIGIT⁺ T cells and BCSCs is a conserved trait of the HER2-positive tumor microenvironment.

Spatial connectivity of the two populations still existed in the peritumoral area of ROI3 (**Figures 4H, I**). The lower subregion showed an enhanced interaction between exhausted CD8A⁺ T cells and CD44⁺CD24⁻ BCSCs (**Figures 4K, L**). In conjunction with the results from tumour-rich areas, these data indicate that the exhausted T cell-BCSC axis is a ubiquitous feature of the microenvironment and remains spatially organized independently of changes in local tumour cell concentration or stromal abundance.

In short, based on our spatial interaction network and matrix analysis, it has been found that CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ BCSCs are in proximity in all areas of the histological microregion. Based on the above data, it can be concluded that exhausted T cells and cancer stem-like cells in HER2-positive breast cancer are in contact.

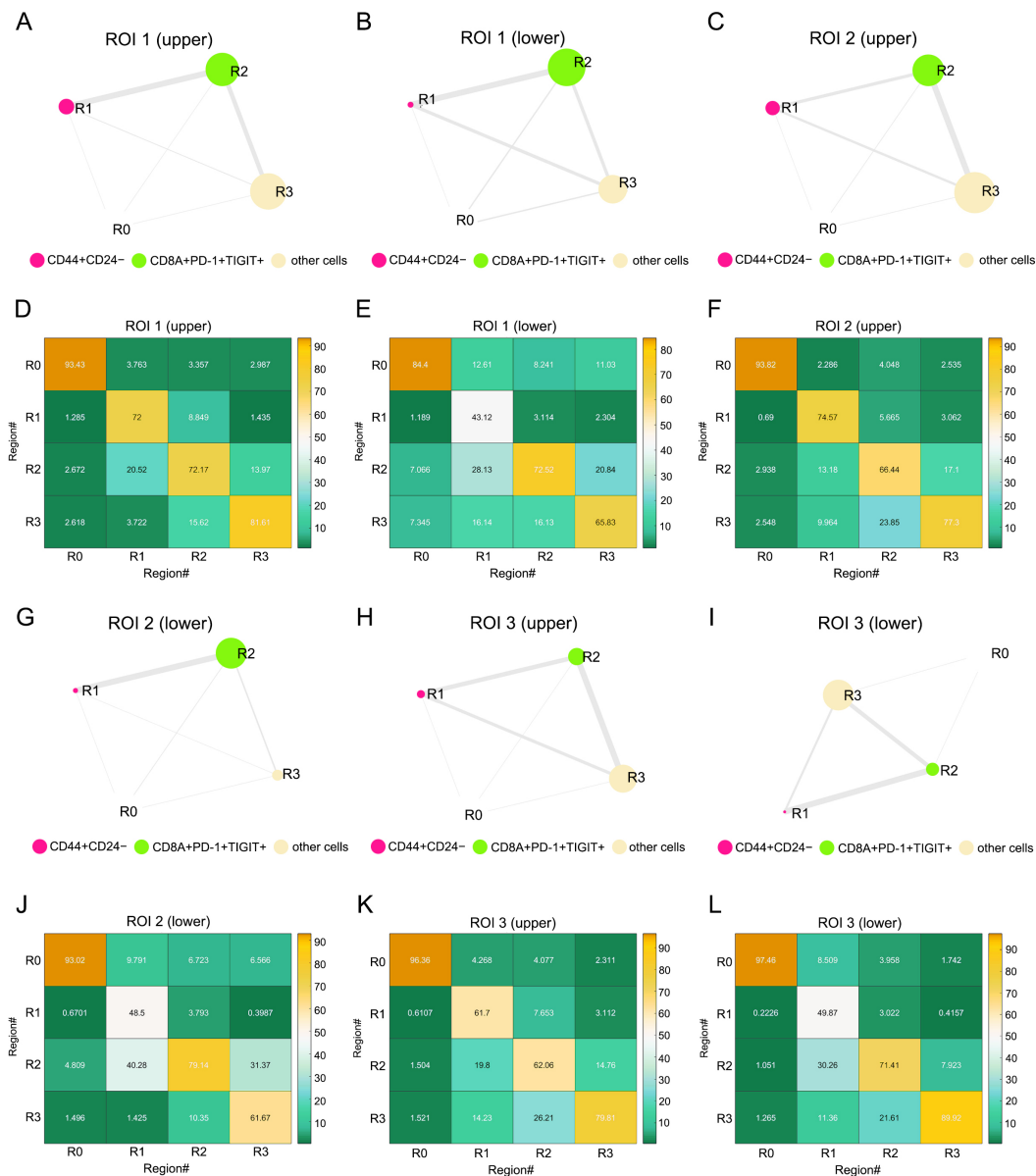


Figure 4. Spatial interaction network analysis further confirms the proximity between CD8A⁺PD-1⁺TIGIT⁺ T cells and CD44⁺CD24⁻ breast cancer stem cells. (A–C, G–I) Spatial interaction networks generated from local regions of ROI1–ROI3 shown in Figure 2. Magenta nodes indicate CD44⁺CD24⁻ BCSCs, fluorescent green nodes indicate CD8A⁺PD-1⁺TIGIT⁺ T cells, and pale-yellow nodes indicate other cells. Node size represents the relative abundance of each cell population, and edge thickness indicates interaction strength. (D–F, J–L) Corresponding spatial interaction matrices. Values and color intensity indicate the degree of spatial proximity between cell populations, with yellow representing stronger interactions and green representing weaker interactions. R1, CD44⁺CD24⁻ BCSCs; R2, CD8A⁺PD-1⁺TIGIT⁺ T cells; R3, other cells; R0, unclassified/background cell populations.

4. Discussion

Multiplex immunofluorescence combined with computational spatial models was used to show that the enrichment of CD8A⁺PD-1⁺TIGIT⁺ T cells is not random but strictly confined to the invasive front of

the tumor-stroma, and they are significantly lacking in the surrounding fibro-adipose tissue. The core of this spatial organisation is the strong link among exhausted T cells and CD44⁺CD24⁻ BCSCs, and this association also occurs in many types of tissue structures. As shown in **Figure 5**, our proposed model of “exhaustion zoning” can divide the tumour microenvironment into multiple areas with differing degrees of immunosuppression. In short, the above results indicate that the BCSC-exhausted T cell axis is a relatively isolated and self-sustaining immunosuppressive area that can lead to treatment failure.

The conventional interpretation of CD8A⁺ T-cell density as a surrogate for active immunity is challenged by the localized exhaustion signature identified in this study ^[13]. Despite their enrichment in invasive regions, these CD8A⁺ T cells predominantly exhibit a PD-1⁺TIGIT⁺ phenotype, indicative of a compromised functional state ^[14]. Therefore, despite a high level of infiltration in HER2-positive tumours, it may not be indicative of immune cell activity but rather a local area of immune suppression. The above observations suggest that, at present, only cell counting is being performed; we need to consider all dimensions of distribution and function to conduct a more comprehensive analysis of immune cell infiltration.

Based on our analysis, there is a non-random spatial proximity between exhausted CD8A⁺ T cells and BCSCs, and this is not due to individual tissue differences. Therefore, the co-occurrence of these two in the high-risk pathological area suggests that cancer stemness and immune exhaustion are not independent phenomena but are functionally related at this site. Thus, such a space is likely to offer a favourable environment for tumour survival under immune pressure. By finding this repeated connection, our research supports the hypothesis that BCSCs can organise a localised immunosuppressive environment and thus protect the regenerative centre of the tumour from effective T-cell-mediated elimination, contributing to the aggressive clinical behaviour of HER2-positive breast cancer ^[15].

The first is the application of space-time analysis to observe changes in the immune microenvironment of tissues at a high resolution in this paper. Non-spatial assays combine signals from various cell types; on the other hand, a spatial framework can identify cellular “neighborhoods” precisely ^[16]. Through triangulation of results from wide-area distribution patterns, local ROI deep dives and network-based proximity metrics, consistent evidence has been provided for the spatial coupling of exhausted T cells and BCSCs. Based on the above data, immunosuppression is inherently a localised phenomenon that presents as a high-risk area of abnormal immune microenvironment rather than an even distribution throughout the whole tumour.

Based on the above data, it is proposed that, in the clinical application of HER2-positive breast cancer treatment, a dual strategy of targeting tumour stemness and reversing immune exhaustion should be adopted simultaneously, rather than focusing on only one of these pathways. Notably, the spatial co-enrichment of CD8A⁺PD-1⁺TIGIT⁺ T cells with CD44⁺CD24⁻ BCSCs has provided a microanatomical basis for exploring combination regimens that integrate anti-HER2 agents, stemness pathway inhibitors, and dual immune checkpoint blockade of PD-1 and TIGIT.

This study has several limitations that should be noted. The cohort size was relatively small, and the spatial distributions reported here await validation in larger, independent patient populations. Additionally, spatial proximity between cell populations does not, by itself, establish direct intercellular communication or causality. Mechanistic dissection through spatial transcriptomics, single-cell RNA sequencing, ligand–receptor interaction modeling, and functional assays will be required in subsequent work. It should also be recognized that although the CD44⁺CD24⁻ immunophenotype is commonly employed as a surrogate for breast cancer stemness, its biological significance in this particular setting would benefit from corroboration using alternative stemness markers and functional validation experiments.

Taken together, our data reveal that CD8A⁺PD-1⁺TIGIT⁺ exhausted T cells preferentially accumulate within invasive pathological microregions of HER2-positive breast cancer, where they reside in consistent spatial

proximity to CD44⁺CD24⁻ BCSCs. These observations provide spatially resolved evidence linking local tumor stemness features with T-cell dysfunction, and point toward this immunosuppressive microenvironment as a potentially important determinant of immune evasion and treatment resistance in HER2-positive disease.

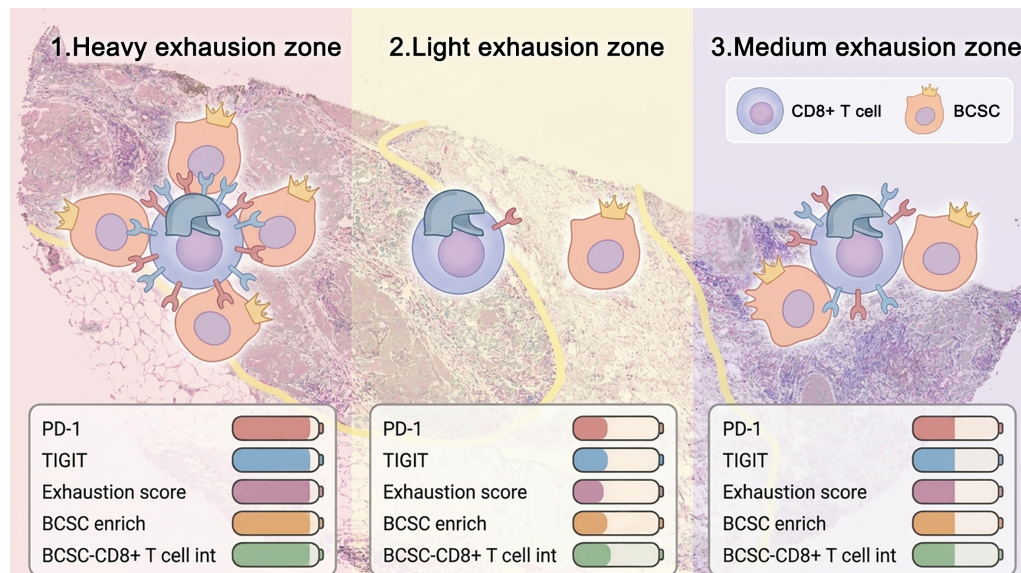


Figure 5. Schematic model illustrating spatially heterogeneous immune-exhaustion niches in HER2-positive breast cancer.

5. Conclusion

In conclusion, this study demonstrates that CD8A⁺PD-1⁺TIGIT⁺ T cells are preferentially enriched in invasive pathological regions of HER2-positive breast cancer and show stable spatial proximity to CD44⁺CD24⁻ breast cancer stem-like cells. These findings suggest that tumor stemness and T-cell exhaustion may be locally coupled within distinct microenvironmental niches. Our results provide spatial evidence for a region-specific immunosuppressive landscape in HER2-positive breast cancer and offer a basis for future investigation into combined therapeutic strategies targeting both cancer stemness and immune exhaustion.

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Disclosure statement

The authors declare no conflict of interest.

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Abnormal Protein Structure Characterization Based on Proteomics and Its Application in Disease Diagnosis

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Abstract: As the key agents of biological processes, proteins' functionality is closely tied to the integrity of their spatial structure. Structural abnormalities can lead to protein dysfunction, which, in consequence, may induce a variety of diseases. Proteomics focuses on the entire proteome. The combination of high-throughput detection techniques and bioinformatics analysis has opened up new possibilities for characterising abnormal protein structures. It also provides a crucial molecular basis for the early diagnosis, classification and prognosis assessment of diseases. This review summarises the core proteomics techniques used to characterise abnormal protein structures. It focuses on the distinguishing features of such structures in fields such as neurodegenerative diseases, cancer and metabolic disorders, as well as their diagnostic applications, and provides a reference for proteomics-based research into abnormal proteins and the precise diagnosis of diseases.

Keywords: Proteomics; Abnormal proteins; Structural characterization; Disease diagnosis; Molecular biomarkers

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1. Introduction

Proteins are the end products of gene expression; their function is determined not only by their amino acid sequence, but also by their secondary, tertiary and quaternary structures. In physiological conditions, proteins rely on molecular chaperones to fold correctly. Multiple regulatory mechanisms, including synthesis, modification, transport and degradation, maintain the structural stability of proteins^[1]. This results in a system of protein stability within the cellular environment, ensuring the normal functioning of all vital processes. When this stability is disrupted, proteins may become misfolded, aggregate abnormally or be mislocalized, resulting in the loss of their original physiological functions and even causing toxic damage, which can subsequently induce pathological changes. Such conditions are also known as protein conformation disorders and are common diseases that threaten human health^[2]. Previous research has mainly

focused on individual proteins, making it difficult to capture dynamic changes in intracellular proteins or to meet the requirements of combined clinical testing. Proteomics focuses on all proteins in the body; by utilising high-throughput technologies such as mass spectrometry and two-dimensional gel electrophoresis, combined with bioinformatics methods, it can systematically analyse protein expression, structure and interactions ^[3]. With the continuous advancement of relevant technologies, the accuracy of detecting abnormal proteins has improved significantly, and proteomics is now widely used in the early screening, precise diagnosis and assessment of treatment efficacy for clinical diseases.

2. Methods of characterising abnormal protein structures based on proteomics

2.1. Laboratory analysis techniques

The core of the characterization of abnormal protein structures is the provision of direct evidence through experimental methods that directly detect the spatial conformation, modification status and aggregation properties of proteins. Recently, analytical techniques based on mass spectrometry (MS), nuclear magnetic resonance (NMR) and cryo-electron microscopy (Cryo-EM) have become mainstream, significantly improving the resolution and efficiency of structural determination.

2.1.1. Mass spectrometry

Mass spectrometry (MS) has become a core technique in proteomics due to its high sensitivity, high resolution and high throughput. MS enables the sequencing of abnormal proteins, the detection of post-translational modifications, the analysis of conformational changes and the identification of aggregation states ^[4]. The main types of MS currently in use include the following:

Cross-Linking Mass Spectrometry (XL-MS) involves adding a cross-linking agent to a protein sample to form covalent cross-links between amino acid residues. The cross-linked peptides are then detected by mass spectrometry and analysed using bioinformatics to determine the spatial distances between amino acid residues. This enables the construction of three-dimensional structural models, allowing for the detection of conformational changes and abnormal interactions within proteins ^[5]. For example, in Alzheimer's disease, this technique can detect cross-linking sites in β -amyloid (A β) and characterise the conformational changes that occur during its aggregation process.

Hydrogen-deuterium exchange mass spectrometry (HDX-MS) utilises the exchange properties of amide hydrogen with deuterium water; by detecting changes in exchange rates via mass spectrometry, it analyzes the secondary structure and conformational dynamics of proteins—regions that are misfolded or unfolded exhibit significantly higher exchange rates than normal regions, enabling rapid differentiation of conformational differences ^[6]. This technique requires only a small sample size and offers rapid detection. It is widely used for the characterization of abnormal proteins associated with tumours, such as the detection of conformational abnormalities in the p53 protein.

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) involves mixing proteins with a matrix; laser excitation generates ions, and molecular weight is determined based on time-of-flight, enabling rapid detection of protein aggregation states. It is suitable for screening for amyloid aggregation, such as the detection of α -synuclein aggregation in Parkinson's disease ^[7]. MS/MS allows for further analysis of amino acid sequences, identifies sites of structural abnormalities, and provides a basis for precise identification.

2.1.2. Nuclear magnetic resonance (NMR) technology

Nuclear magnetic resonance (NMR) technology utilises the resonance of atomic nuclei in a magnetic field. By measuring the chemical shifts of nuclei such as hydrogen, carbon and nitrogen, NMR characterises the secondary and tertiary structures, and conformation dynamics, of small-molecule proteins (molecular weight < 50 kDa) ^[8]. This technology enables real-time monitoring of protein folding processes and captures intermediate states of misfolding. For example, in prion diseases, it can detect the transition of prion protein (PrP) from its normal conformation (PrP^C) to its pathogenic conformation (PrP^{Sc}), thus characterising the underlying molecular mechanisms.

Developments in High-Field NMR technology have broadened applications. This technique can resolve the structures of protein complexes, but it is subject to limitations such as high sample consumption, lengthy analysis times, and difficulties in resolving macromolecules. For this reason, it is most commonly employed in conjunction with MS for enhanced accuracy and comprehensiveness in characterization.

2.1.3. Cryo-electron microscopy

Cryo-EM quickly freezes protein samples to liquid nitrogen temperature to preserve their native structure. Two-dimensional images are then captured using an electron microscope, and these are combined with three-dimensional reconstruction techniques to construct a three-dimensional structural model. This method offers high resolution, no requirement for crystallisation, and minimal sample usage, and is especially suitable for the characterization of macromolecular proteins and aggregates ^[9,10]. This is a key method for characterising the structure of abnormal protein aggregates. In Alzheimer's disease, it enables the analysis of the structure of A β aggregates, clarifying their aggregation patterns and mechanisms of toxicity. Furthermore, in Parkinson's disease, it allows the structure of α -synuclein aggregate fibres to be resolved, providing direct evidence for research into the disease's pathogenic mechanisms. Last but not least, two-dimensional gel electrophoresis (2-DE), as a conventional technique, separates proteins of different molecular weights and isoelectric points by combining isoelectric focusing with SDS-PAGE electrophoresis. It enables preliminary screening by exploiting the differences in migration rates between abnormal and normal proteins, and when combined with mass spectrometry for identification, provides initial clues for characterization ^[11].

2.2. Bioinformatics prediction methods

Developments in bioinformatics have improved tools for protein structure prediction based on amino acid sequences. Presently, these tools can quickly predict secondary and tertiary structures, support the characterization of abnormal proteins, and compensate for limitations in laboratory techniques. Commonly used methods are as follows:

Protein structure prediction tools such as AlphaFold and RosettaFold analyse amino acid sequences using deep learning algorithms. These tools can predict three-dimensional structures with an accuracy of over 90%, enabling the rapid identification of the conformational features and abnormal sites of abnormal proteins ^[12]. For example, AlphaFold can be used to predict the three-dimensional structure of the mutant p53 protein, thus elucidating the mechanism of its loss of function; it can also predict the structure of A β , helping to analyse the structural changes that occur during the assembly process.

Misfolding prediction tools, like TANGO and AGGRESCAN, are designed to analyse amino acid sequences. They predict the likelihood of misfolding and identify aggregation domains, enabling the rapid screening of high-risk proteins. This technology is widely used in the prediction of proteins associated with

neurodegenerative diseases. Examples include the prediction of abnormalities in the huntingtin protein in Huntington's disease and the SOD1 protein in amyotrophic lateral sclerosis ^[13].

NetPhos and NetGlyc are post-translational modification prediction tools that can forecast phosphorylation, glycosylation and other modification sites, and support the analysis of structural abnormalities resulting from modification defects ^[14]. Taking examples, when predicting phosphorylation sites on the Tau protein, the tool can be used to identify conformational abnormalities caused by hyperphosphorylation; it can also be employed to predict phosphorylation sites on the EGFR protein, to analyse the association between its mutations and the development of tumours.

Bioinformatics-based predictions have the advantages of being fast, efficient and cost-effective. It enables large-scale protein screening, but the results must be confirmed through experimental results to ensure accuracy.

3. Applications of abnormal protein structures in disease diagnosis

3.1. Neurodegenerative diseases

Core pathological features of neurodegenerative diseases include the misfolding and aggregation of specific proteins, resulting in insoluble amyloid deposits. This subsequently leads to neuronal damage and death ^[15]. Traditional diagnosis of this type of disease has been heavily reliant on clinical symptoms and imaging tests. This approach lacks specific molecular biomarkers, which makes early diagnosis particularly challenging. Proteomics technology, however, has opened up new paths for its early diagnosis.

Alzheimer disease (AD)'s core pathological features are the abnormal aggregation of A β and the hyperphosphorylation of tau protein ^[16]. A β is produced by the abnormal cleavage of the APP protein; in its normal state, it exists as a soluble monomer, but when abnormal, it misfolds and aggregates into amyloid fibrils, which deposit in the cerebral cortex and hippocampus. Overphosphorylation of the tau protein leads to the formation of neurofibrillary tangles, which disrupt the neuronal cytoskeleton. In the study, the misfolded intermediate state of A β was identified as a key factor in the early onset of AD and may serve as a potential diagnostic biomarker. Overexpression of Ser396 and Ser404 is associated with disease progression and can be used to assess disease status ^[17].

Parkinson's disease (PD) is characterised by the misfolding and aggregation of α -synuclein (α -syn) into Lewy bodies. α -syn aggregation occurs in three stages: monomers, oligomers and fibrils, with oligomers being the primary toxic form responsible for damage to dopaminergic neurons ^[18]. The levels of α -syn oligomers in the cerebrospinal fluid of Parkinson's disease patients are significantly higher than in healthy individuals and may serve as a biomarker for early diagnosis. The N-terminal and C-terminal regions are key sites for misfolding, and mutations in the relevant amino acids accelerate aggregation, making them useful for genetic risk assessment.

Amyotrophic lateral sclerosis (ALS) is characterised by the misfolding and aggregation of proteins such as TDP-43 and SOD1, which form inclusion bodies ^[19]. Mutations in the SOD1 gene lead to misfolding of the protein, resulting in oxidative stress and neuronal toxicity; abnormal modification of TDP-43 disrupts RNA metabolism. Research indicates that abnormal TDP-43 interactions can serve as a diagnostic biomarker, while the intermediate state content of misfolded SOD1 can be used to monitor treatment efficacy.

3.2. Tumour

Structural abnormalities in proteins are closely linked to the development and progression of cancers. The activation of proto-oncogenes and the inactivation of tumour suppressor genes are often caused by changes in protein conformation, abnormal post-translational modifications, or abnormal aggregation ^[20]. Through proteomic characterization of abnormal protein structures, tumour-specific molecular biomarkers can be identified. This provides a basis for early screening, precise classification and prognostic assessment, thereby advancing precision medicine.

The p53 protein is a major tumour suppressor, and its dysfunction is associated with more than 50% of human solid tumours. Normal wild-type p53 regulates the cell cycle and induces apoptosis ^[21]; mutations in the p53 gene can lead to misfolding of the protein, loss of its tumour-suppressing function, and even the acquisition of tumour-promoting activity. NMR and mass spectrometry analyses have shown that the core DNA-binding domain of p53 is a mutation hotspot. Following mutation, its ability to bind to DNA is reduced and its conformational stability is diminished, leading to the formation of insoluble aggregates. HDX-MS can rapidly distinguish between wild-type and mutant p53 based on differences in hydrogen-deuterium exchange rates. Results indicate that mutation sites such as R175H and R273H are associated with rapid disease progression and poor prognosis and can therefore be used for prognostic assessment.

The epidermal growth factor receptor (EGFR) is a critical therapeutic target in cancer treatment, and structural abnormalities in this receptor are associated with non-small cell lung cancer, breast cancer and other malignancies ^[22]. While the native EGFR exists as a monomer, ligand binding induces dimerisation, which activates downstream signalling pathways. Mutations such as the deletion of exon 19 and the L858R mutation in exon 21 can alter its conformation, leading to sustained activation in the absence of ligands and driving tumour proliferation. Cryo-EM analysis reveals that EGFR mutant dimers are more stable, with conformational changes in the kinase domain being key to sustained activation ^[23]; XL-MS detection has found that phosphorylation levels in the tyrosine kinase domain are significantly elevated, which can serve as a biomarker for diagnosis and the assessment of targeted therapy efficacy.

Researchers from Fudan University analysed the plasma proteomes of 53,026 individuals and found that over 650 proteins are associated with at least 50 diseases. GDF15 emerged as a key biomarker for the diagnosis and prognosis of various tumours, whilst EDA2R, NTproBNP and others also demonstrated good diagnostic performance ^[24]. Furthermore, mutations in splicing factors within tumours lead to abnormal splicing of genes such as CD44 and VEGFR; the resulting abnormal protein isoforms can serve as diagnostic markers for tumour invasion, metastasis and drug resistance, and mass spectrometry can be used to detect them with high precision.

3.3. Metabolic disorders

Metabolic disorders (such as diabetes, obesity and fatty liver disease) are closely linked to metabolic disturbances caused by abnormal protein structures; proteomic characterization can elucidate the underlying molecular mechanisms, thus providing a basis for diagnosis and intervention.

The core pathological features of type 2 diabetes mellitus (T2DM) are insulin resistance and insufficient secretion, which are associated with structural abnormalities in the insulin protein and the insulin receptor (IR). Mass spectrometry and NMR analyses have shown that misfolding of the insulin protein reduces its activity, whereas abnormal phosphorylation of the tyrosine kinase domain of the IR leads to conformational changes,

preventing the activation of downstream signalling pathways and resulting in insulin resistance ^[25]. HDX-MS analysis has revealed that the cleavage of disulfide bonds between the A and B chains of insulin leads to misfolding, forming inactive aggregates; the levels of these aggregates correlate with disease progression and may serve as diagnostic biomarkers. Furthermore, abnormal phosphorylation at Tyr1162 and Tyr1163 on the IR is associated with insulin resistance and can be utilised for disease assessment.

Abnormal protein structures are closely associated with obesity and adipocyte differentiation; peroxisome proliferator-activated receptor gamma (PPAR γ) is a key regulatory factor, whose conformational changes influence ligand-binding capacity and regulate adipocyte differentiation and lipid metabolism. Cryo-EM analysis reveals that an abnormal conformation of the PPAR γ ligand-binding domain leads to excessive activation, promoting excessive adipocyte differentiation; XL-MS detection has found that abnormal interactions with co-activators can serve as diagnostic markers and intervention targets for obesity.

4. Conclusion

Proteomics technologies provide a novel technical foundation for the systematic characterization of abnormal protein structures. Through the combined application of experimental detection and bioinformatics-based prediction, it is possible to comprehensively analyse the conformational changes, modification status and aggregation characteristics of abnormal proteins. Abnormal protein structures exhibit specific characteristics in a wide range of diseases, including neurodegenerative disorders, cancer and metabolic diseases. Characterising these structures not only aids in elucidating the pathogenic mechanisms of these diseases but also provides important molecular biomarkers for early diagnosis, disease classification and prognosis assessment. With continuous technological innovation and deepening research, their application in disease diagnosis will become increasingly widespread. This is expected to drive major breakthroughs in precision diagnosis and treatment, thereby providing stronger safeguards for human health.

Disclosure statement

The authors declare no conflict of interest.

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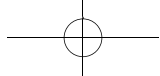
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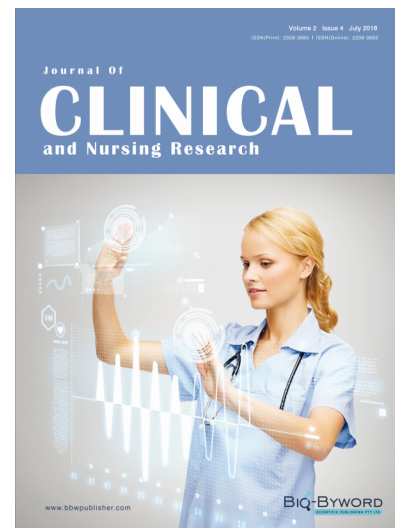
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