

Oncology Treatment Discovery

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Oncology Treatment Discovery

Focus and Scope

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Evaluation of the Effect of Open Fistula Removal in the Surgical Treatment of Anal Fistula

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Abstract: *Objective:* To analyze the treatment effect of open fistulotomy on patients with anal fistula. *Methods:* A total of 42 patients with anal fistula who visited the hospital from March 2021 to March 2024 were selected as samples and grouped using a random number table. The observation group received open fistulotomy, while the control group received conventional treatment. The differences in efficacy, surgical indicators, inflammatory factors, pain scores, and complications were compared. *Results:* The cure rate of patients with anal fistula in the observation group was higher than that in the control group, and the infection rate and recurrence rate were lower than those in the control group ($P < 0.05$). The surgical operation time, wound healing time, and hospital stay in the observation group were shorter than those in the control group ($P < 0.05$). There was no significant difference in intraoperative blood loss between the observation group and the control group ($P > 0.05$). The interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), and visual analog scale (VAS) scores in the observation group were lower than those in the control group ($P < 0.05$). The complication rate of patients with anal fistula in the observation group was lower than that in the control group ($P < 0.05$). *Conclusion:* Open fistulotomy for the treatment of patients with anal fistula can reduce inflammatory reactions, optimize surgical indicators, reduce pain, and is safe and efficient.

Keywords: Anal fistula; Open fistulotomy; Efficacy

Online publication: January 3, 2025

1. Introduction

Anal fistula is a common anal lesion, often induced by nonspecific infection. It consists of a fistula wall, internal opening, and external opening, with the pathological feature of having one internal opening and one or more external openings^[1]. Once an anal fistula forms, it is difficult to heal spontaneously. If not treated promptly, it can lead to secondary infectious purulent discharge, increasing the risk of complications such as anal incontinence, mental lethargy, and decreased body mass, thereby increasing the psychological and physiological stress on the patient. Currently, surgical procedures are commonly used to treat anal fistulas in clinical practice. Opening the wound in the form of an incision fistula can shorten the healing time. However, conventional surgical treatment

for anal fistulas carries a high risk of postoperative complications and the possibility of pseudo-healing. Open fistula resection is a new surgical approach that involves incision of the fistula and opening of the wound, which can shorten wound healing time. This article explores the efficacy of open fistula resection using a sample of 42 patients with anal fistulas who visited the hospital from March 2021 to March 2024.

2. Materials and methods

2.1. Materials

A total of 42 patients with anal fistula who visited the hospital from March 2021 to March 2024 were selected as samples and randomly divided into groups using a random number table. The baseline data of patients with anal fistula in the observation group (Group A) were compared with those in Group B, with $P > 0.05$ (Table 1).

Table 1. Baseline data analysis of patients with anal fistula

Group	<i>n</i>	Gender (%)		Age (years)		Duration of illness (days)	
		Male	Female	Range	Average	Range	Average
Group A	21	12 (57.14)	9 (42.86)	11–72	37.89 ± 2.42	3–15	11.89 ± 2.42
Group B	21	13 (61.90)	8 (38.10)	11–73	37.91 ± 2.39	4–16	11.91 ± 2.39
χ^2/t	-	0.0988		0.0269		0.0269	
<i>P</i>	-	0.7532		0.9786		0.9786	

2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) Meet the criteria for anal fistula in the “Treatment Guidelines for Perianal Abscess, Anal Fistula, and Rectovaginal Fistula” [2]; (2) No abnormalities detected in preoperative anal examination; (3) Informed consent obtained; (4) Stable vital signs.

Exclusion criteria: (1) Blood system disorders; (2) Coagulation disorders; (3) Acute infectious diseases; (4) Cardiovascular and cerebrovascular diseases; (5) Cognitive impairments.

2.3. Treatment method

2.3.1. Observation group

Patients were switched to a liquid diet one day before surgery. Enema with soapy water was prepared 4–6 hours before the operation. If imaging suggested a high anal fistula, a 40% iodinated oil contrast examination was performed. Patients were taken into the operating room and underwent lumbar anesthesia. They were assisted to maintain a jackknife position. White gauze was prepared and inserted into the anal canal. A small amount of methylene blue was injected through the external opening of the fistula, and the direction of the fistula was determined based on the staining of the gauze. A slotted probe was inserted through the external opening and advanced to the deepest part of the fistula. The skin, subcutaneous tissue, and outer wall of the fistula were incised sequentially until the apical fistula area was reached. The fistula was then resected, followed by the removal of adjacent skin and subcutaneous tissue. Emphasis was placed on controlling bleeding from the surgical incision. After achieving hemostasis, the incision skin was trimmed and sutured. For patients with high anal fistulas, a distal incision was performed, and the incision length was adjusted based on the length of the fistula. A rubber band was hung at the proximal end for drainage after completing the surgery. The incision dressing was changed 24

hours after the operation. Two days postoperatively, the wound surface was cleaned with potassium permanganate solution, followed by instructions for the patient to sit in a sitz bath. Three days after surgery, antibiotics were administered prophylactically. Patients were consulted about the presence of subjective symptoms, and the growth of granulation tissue on the wound surface was evaluated. Any abnormalities were promptly addressed.

2.3.2. Control group

The patients underwent conventional fistula resection in the jackknife position. Lumbar anesthesia was administered, and the fistula along with adjacent tissues was separated. The diseased tissue was then resected until healthy tissue was exposed at the fistula site. The incision was closed using absorbable sutures. Postoperatively, patients were instructed to perform medicinal sitz baths until complete wound healing and then discontinued.

2.4. Observation indicators

- (1) Efficacy criteria: If the post-operative vital signs of anal fistula patients remain stable, surgical wounds have healed, and there are no symptoms such as bleeding or edema, it is considered a complete recovery. If postoperative anal fistula patients experience abnormal fluctuations in vital signs, rough surgical wounds, presence of symptoms such as bleeding or edema, and follow-up visits reveal new external fistula openings, it is considered a recurrence. If there is a post-operative incision infection, it is noted as an infection.
- (2) Surgical indicators: Record surgical operation time, wound healing time, hospital stay, intraoperative blood loss, and other relevant indicators.
- (3) Inflammatory factors and pain scores: Detection of IL-4, IL-6, IL-10, and other indicators using enzyme-linked immunosorbent assay (ELISA). VAS (Visual Analog Scale) ranging from 0–10, where the score is directly proportional to the pain felt by the patient with an anal fistula.
- (4) Complications: Record complications such as incision infection, anal deformation, anal displacement, and others.

2.5. Statistical analysis

Data processing will be done using SPSS 21.0 software. Count data will be recorded as percentages (%) and analyzed using the chi-square test (χ^2 test). Measurement data will be recorded as mean \pm standard deviation (SD) and analyzed using the *t*-test. Statistical significance will be set at $P < 0.05$.

3. Results

3.1. Analysis of therapeutic effects on patients with anal fistula

The cure rate in the observation group was higher than that in the control group, while the infection rate and recurrence rate were lower than those in the control group ($P < 0.05$) (Table 2).

Table 2. Comparison of therapeutic effects on patients with anal fistula (*n*, %)

Group	Cure	Infection	Recurrence
Observation group (<i>n</i> = 21)	20 (95.24)	1 (4.76)	0 (0.00)
Control group (<i>n</i> = 21)	11 (52.38)	6 (28.57)	4 (19.05)
χ^2	9.9765	4.2857	4.4211
<i>P</i>	0.0016	0.0384	0.0355

3.2. Analysis of surgical indicators for patients with anal fistula

The observation group had shorter surgical operation time, wound healing time, and hospital stay compared to the control group, with $P < 0.05$. However, there was no significant difference in intraoperative blood loss between the observation group and the control group, with $P > 0.05$. See **Table 3** for details.

Table 3. Comparison of surgical indicators for patients with anal fistula (mean \pm SD)

Group	Surgical operation time (min)	Wound healing time (d)	Hospital stay (d)	Intraoperative blood loss (mL)
Observation group ($n = 21$)	12.39 \pm 1.25	8.28 \pm 1.42	14.88 \pm 2.11	97.11 \pm 3.69
Control group ($n = 21$)	27.44 \pm 1.81	14.33 \pm 2.09	20.69 \pm 2.99	98.06 \pm 3.72
χ^2	31.3535	10.9724	7.2754	0.8309
P	0.0000	0.0000	0.0000	0.4110

3.3. Analysis of inflammation indices and pain score indices in patients with anal fistula

After treatment, the levels of IL-4, IL-6, and IL-10, as well as the VAS score, were lower in the observation group compared to the control group, with $P < 0.05$. See **Table 4** for details.

Table 4. Analysis of inflammation indices and pain score indices in patients with anal fistula (mean \pm SD)

Group	IL-4 (ng/mL)		IL-6 (ng/mL)		IL-10 (ng/mL)		VAS (points)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group ($n = 21$)	134.25 \pm 2.48	40.01 \pm 1.88	246.84 \pm 2.78	46.44 \pm 1.91	57.44 \pm 2.44	40.21 \pm 1.25	3.36 \pm 1.25	1.22 \pm 0.18
Control group ($n = 21$)	133.29 \pm 2.51	72.44 \pm 2.09	246.79 \pm 2.81	114.39 \pm 2.19	57.36 \pm 2.43	46.28 \pm 1.69	3.39 \pm 1.27	2.57 \pm 0.21
t	1.2468	52.8657	0.0580	107.1568	0.1065	13.2329	0.0771	22.3673
P	0.2197	0.0000	0.9541	0.0000	0.9157	0.0000	0.9389	0.0000

3.4. Analysis of complication indexes of patients with anal fistula

The complication rate of the observation group was lower than that of the control group ($P < 0.05$) (**Table 5**).

Table 5. Analysis table of complication indexes of patients with anal fistula (n , %)

Group	Incision infection	Anal degeneration	Anal displacement	Incidence rate
Observation group ($n = 21$)	1 (4.76)	0 (0.00)	0 (0.00)	1 (4.76)
Control group ($n = 21$)	3 (14.29)	2 (9.52)	1 (4.76)	6 (28.57)
χ^2	-	-	-	4.2857
P	-	-	-	0.0384

4. Discussion

Anal fistulas are predominantly found in the male population aged between 20 and 40 years, with various contributing factors such as perianal infection, perianal abscess, and surgical procedures^[3]. Once formed, anal fistulas often persist and can lead to complications like mental lethargy and bowel dysfunction. A significant proportion of patients with anal fistulas have inadequate understanding of their condition, resulting in poor treatment adherence and delayed medical intervention, which can exacerbate the fistula's severity. Therefore, it is imperative to adopt effective treatment strategies for managing anal fistulas^[4]. Most patients with anal fistulas are unable to achieve spontaneous resolution, necessitating surgical intervention. However, conventional surgical approaches for anal fistulas carry a high risk of complications and a certain degree of recurrence after surgery. In recent years, open fistulotomy has emerged as a treatment option for patients with anal fistulas. This surgical technique not only effectively addresses the internal opening but also allows for precise localization and removal of the internal opening along with adjacent inflamed tissues, thereby reducing the likelihood of postoperative recurrence^[5]. Furthermore, open fistulotomy is characterized by minimal pain and simplicity in execution. The procedure involves minimal damage to the anal sphincter muscles, preserving the patient's anorectal function^[6]. These advantages make open fistulotomy a viable and preferred surgical option for the management of anal fistulas, offering patients a safer and more effective treatment pathway.

Based on the data analysis in this paper, the cure rate of anal fistula patients in the observation group was higher than that in the control group, while the infection rate and recurrence rate were lower than those in the control group, with $P < 0.05$. The reason for this is that open fistulotomy treatment involves removing or trimming the mucosa in the adjacent area of the internal opening, followed by closing the internal opening, fully stopping bleeding in the wound and suturing the surgical incision. This can reduce local tissue damage and postoperative infection events, which is beneficial for shortening the incision healing time, thus resulting in a higher postoperative cure rate^[7]. Another set of data showed that the operation time, wound healing time, and hospital stay in the observation group were shorter than those in the control group, with $P < 0.05$. However, there was no significant difference in intraoperative blood loss between the observation group and the control group, with $P > 0.05$. The reason for this is that open fistulotomy treats the lesion by identifying it through the primary internal opening and then removing the infected tissue adjacent to the perianal area and anal sinuses, which can eliminate the infection. Additionally, the simplicity of the open surgical procedure and its advantages in wound management can reduce damage to the anal sphincter caused by mechanical manipulation, resulting in minimal intraoperative bleeding and shortened operation time, which is conducive to postoperative recovery of anal function^[8]. Another set of data indicated that the levels of IL-4, IL-6, and IL-10, as well as the VAS score, were lower in the observation group compared to the control group, with $P < 0.05$. The reason for this is that patients with anal fistula who undergo conventional surgical treatment may experience inflammatory reactions due to mechanical stimulation, leading to increased release of IL-4, IL-6, and IL-10. If timely anti-infection intervention is not provided, it can affect the recovery of anal function^[9]. Performing open fistulotomy is a simple procedure that involves removing the fistulous tract and further trimming the internal opening and adjacent mucosal tissue. Completing procedures such as internal opening closure, hemostasis, and suturing under direct vision can reduce trauma to the affected area and shorten wound healing time. This is beneficial for reducing physiological stress responses, leading to decreased release of inflammatory factors and increased pain threshold^[10].

The final set of data showed that the complication rate in the observation group was lower than that in the control group, with $P < 0.05$. It has been demonstrated that open fistulotomy has high safety. Upon analysis, the

adoption of open fistulotomy for treatment can repair the mucosal tissue adjacent to the internal opening, laying a good foundation for wound healing. Additionally, open surgery allows for complete removal of the lesion and adequate hemostasis, thereby reducing the rate of postoperative complications^[11]. During the actual treatment of anal fistula patients with open fistulotomy, the following considerations should be noted:

- (1) Maintain a bland diet: Increase intake of high-fiber foods, avoid spicy foods such as ginger, garlic, onions, and alcoholic beverages, and emphasize dietary hygiene management. Furthermore, patients are advised to consume more fresh fruits and vegetables after surgery to prevent constipation and diarrhea, as watery stools can easily remain in the anal sinus area, increasing the risk of postoperative infection.
- (2) Develop bowel habits: Patients are instructed not to defecate within 24 hours after anorectal surgery; after 24 hours, it is recommended to defecate 1–2 times per day.
- (3) Maintain perianal hygiene: Cleanse properly after defecation, and sit in a medicated bath as prescribed by the doctor to avoid fecal irritation and wound contamination. Additionally, patients with anal fistulas should perform local massage after defecation to promote local blood circulation and shorten wound healing time.
- (4) Increase rest time: Reduce activity during the early postoperative period to prevent postoperative bleeding and alleviate local anal symptoms. Furthermore, patients are advised to avoid sitting for long periods, limiting each sitting session to within 30 minutes, avoid long-term cycling and driving, and reduce the frequency of staying up late to shorten the recovery time of anal fistulas.
- (5) Exercise anal function: Guide patients with anal fistulas to actively perform anal lifting exercises after surgery to prevent anal complications and promote the recovery of anal physiological function. In summary, the treatment of anal fistula patients with open fistulotomy can shorten the recovery time, improve the cure rate, reduce inflammatory reactions, and decrease complications, demonstrating its promotional value.

Disclosure statement

The authors declare no conflict of interest.

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Comprehensive Bioinformatics Evaluation of CTNNB1 as a Diagnostic, Therapeutic and Prognostic Biomarker in Liver Hepatocellular Carcinoma

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Abstract: Liver Hepatocellular Cancer (LIHC) is a fatal disease, that keeps rigorous to therapeutic approach. This study explores the expression and promoter methylation of CTNNB1 and survival analysis, to extricate its role in LIHC progression, prognosis, and therapeutic approaches. Employing UALCAN, the study examined upregulation in CTNNB1 expression in LIHC as compared to normal samples explained its role in LIHC progression. Further analysis, stratified by patient's age, gender, race, and pathological stages, revealed upregulation across all variables. Analysis of promoter methylation level of CTNNB1 in LIHC revealed hypomethylation, acknowledging upregulation of expression. Moreover, survival analysis of CTNNB1 using a KM plotter demonstrated its prognostic significance, as CTNNB1 overexpression results in the worst overall survival (OS) and vice versa. Validation via GEPIA2 affirmed elevated expression level of CTNNB1 in LIHC, further establishing its correlation with unfavorable survival outcomes. Furthermore, pathway enrichment analysis utilizing the STRING and DAVID tool identified association with genes implicated in essential signaling processes such as the Wnt signaling pathway revealing its role in LIHC progression. Subsequently, Genetic mutation analysis performed using cBioPortal demonstrated a 10% mutation of CTNNB1 in LIHC, indicating that alteration in the gene has a critical role in the development of LIHC. In conclusion, this comprehensive analysis highlights the significance of CTNNB1 upregulation in LIHC progression and its capability as a prognostic biomarker, offering valuable insight for developing targeted therapeutic strategies.

Keywords: CTNNB1; LIHC; Biomarker; Wnt signaling pathway

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1. Introduction

Cancer is a prominent health and economic issue globally, with high human mortality. As stated by studies, in 2020

about 19.3 million new cases and 10 million cancer-related deaths were reported ^[1-4]. There are numerous types of cancer, liver cancer is 5th most common with 905,677 cases and the second highest 830,180 mortalities worldwide in 2020 ^[1,5]. Liver hepatocellular cancer (LIHC) accounts for 80% of these cases ^[6]. Alcohol consumption, hepatitis B, hepatitis C and metabolic infection are key risk factors of LIHC ^[7-9]. It is a heterogeneous disease and is often diagnosed at advanced stages, which brings problems in treatment ^[7,10]. For LIHC early stages, the treatment includes surgery, liver transplant and immunotherapy ^[11]. While advanced stages of LIHC demonstrate a response to atezolizumab and bevacizumab combined therapy ^[12]. However, it has a 70% recurrence rate and hardly 50% of LIHC patients outlive 5 years after surgery ^[13,14]. Drug resistance, economic burden, less efficient target therapy, high recurrence, and late diagnosis pose challenges to treating LIHC ^[15-18]. Therefore, it is an urgent need to identify diagnostic, prognostic and therapeutic biomarkers to tackle challenges in LIHC treatment.

In recent years, signal transduction pathway activation has been revealed to play a role in LIHC, of these pathways Wnt/ β catenin pathway activation is reported to have a role in the prognosis and development of LIHC ^[19-21]. Catenin beta 1 (CTNNB1) is a key gene located on chromosome 3p22.1 and codes in the Wnt/ β catenin pathway to act as an intracellular signal transducer ^[22,23]. E-cadherin is directly connected to β -catenin and forms an adhesion complex, phosphorylation degrades this complex. This complex maintains cell functions and cell-cell adhesion. Because of phosphorylation, β -catenin dislocates to the nucleus, and activation of Wnt target genes related to cell proliferation, cell cycle and carcinogenesis is triggered ^[24-27].

Because of lifestyle and risk factors in different geographical regions, variations in genetic mutation are identified in liver cancer. As Asia-Pacific region has a low number of LIHC as compared to the eastern Asia region with respect to HCV and HBV rates. Similarly, CTNNB1 mutation is lower in Asia and higher in Europe and America ^[28-30]. CTNNB1 is identified to be mutated in 20 to 40 percent of liver cancer. CTNNB1 mutation is also associated with the progression of different human cancers ^[29,31,32]. CTNNB1 is also associated with irruption and poor prognosis in LIHC ^[24]. All of these studies identified the role of CTNNB1 in the progression of LIHC. But to our knowledge, the role of CTNNB1 as a diagnostic, therapeutic and prognostic biomarker is yet to be identified. Therefore, this study aimed to the bioinformatics analysis of CTNNB1 in LIHC.

2. Material and method

2.1. UALCAN

UALCAN is a user-friendly online software based on The Cancer Genome Atlas (TCGA), employed for gene expression analysis across different tumors ^[33]. This study analyzed the expression and promoter methylation level of CTNNB1 in LIHC using the UALCAN database. The study employed UALCAN to execute analysis in LIHC and normal samples, together with different variables such as patient's age, gender, race and individual cancer stage.

2.2. Kaplan-Meier plotter

Kaplan-Meier (KM) plotter is an online tool that is utilized to verify the survival curve of the specified gene in cancer ^[34]. The study evaluates the OS of LIHC patients affected by CTNNB1 expression by Survival analysis using a KM plotter. A *p*-value less than 0.05 is considered statistically significant and the hazard ratio is calculated with a 95% interval.

2.3. GEPIA2

Gene Expression Profiling and Interactive Analysis version 2 (GEPIA2) is an online server that is used for gene expression and prognostic analysis ^[35]. We also employed GEPIA2 to perform expression and survival analysis of CTNNB1 in LIHC. This study implemented this technology in the box stage and sample-based analysis of CTNNB1 in LIHC. The study also examined survival analysis using the survival module of GEPIA2.

2.4. STRING database

Protein-protein interaction (PPI) networks are constructed utilizing an online database, Search Tool for the Retrieval of Interacting Genes (STRING) ^[36]. This study constructed the PPI network of CTNNB1 using the STRING database.

2.5. DAVID

Pathway enrichment analysis is performed using an online tool, Database for Annotation, Visualization, and Integrated Discovery (DAVID) ^[37]. This study uses the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) pathway enrichment analyses. Cellular component (CC), molecular function (MF), and biological process (BP) are three main categories of GO analysis.

2.6. cBioPortal

Genetic alteration of specific gene is analyzed utilizing an online tool cBioPortal. This study utilized genetic mutation of CTNNB1 in LIHC using cBioPortal.

3. Results

3.1. Analysis of CTNNB1 expression in LIHC

First, the study analyzed the expression of ETNB1 in LIHC and normal samples using the UALCAN database. The study analyzed that CTNNB1 was significantly upregulated in LIHC samples as compared to normal samples (**Figure 1**). This study coincides with previous studies that the upregulation of genes leads to the progression of cancer ^[38,39]. Hence, the upregulation of CTNNB1 has an association with the progression of LIHC.

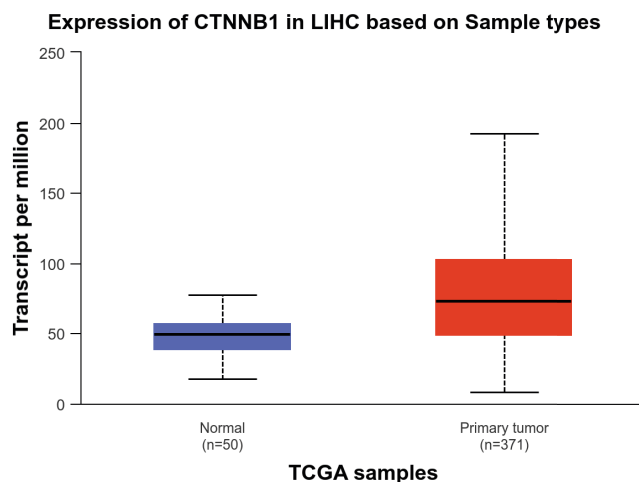


Figure 1. The expression analysis of CTNNB1 in LIHC and normal control sample using the UALCAN database.

3.2. Expression analysis of CTNNB1 in LIHC categorized according to different variables

Following the analysis, the study analyzed CTNNB1 expression in LIHC categorized according to different variables such as patient age, patient gender, patient race and individual pathological stages. First, the study examined CTNNB1 expression in LIHC based on pathological stages. The study evaluated that CTNNB1 was significantly upregulated in each of this individual cancer stages (**Figure 2A**). After that, the study evaluated that CTNNB1 expression was significantly upregulated in LIHC patients of different races (**Figure 2B**). Furthermore, the study investigated significant upregulation in LIHC patient's different age group and gender (**Figure 2C** and **Figure 2D**). Altogether, based on all these results, CTNNB1 has a role in the proliferation of LIHC.

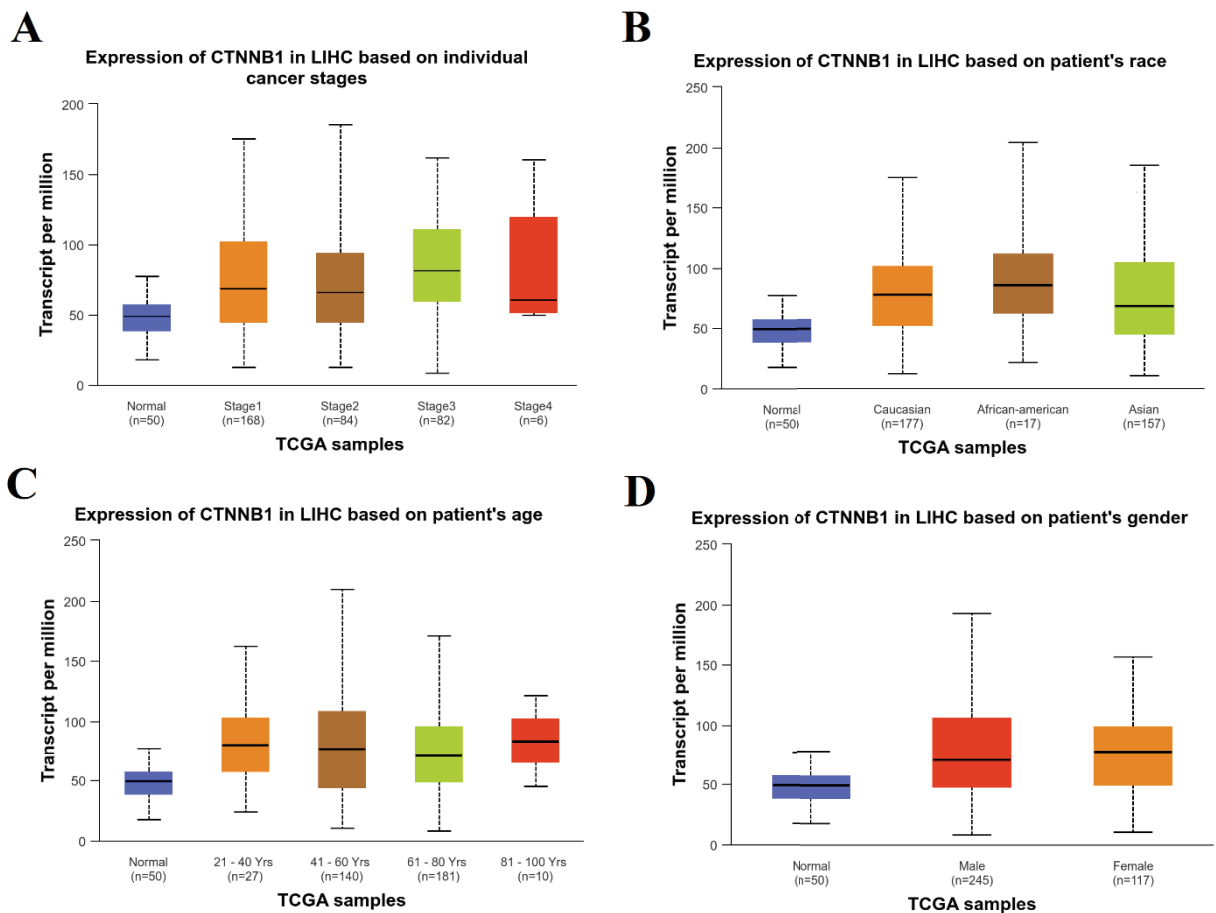


Figure 2. (A) Expression analysis of CTNNB1 in LIHC patient's individual cancer stage; (B) Expression analysis of CTNNB1 in LIHC patient's race; (C) Expression analysis of CTNNB1 in LIHC patient's age group; (D) Expression analysis of CTNNB1 in LIHC patient's gender.

3.3. Analysis of CTNNB1 promoter methylation level in LIHC and normal control sample

The study analyzed CTNNB1 promoter methylation levels in LIHC and normal control samples utilizing the UALCAN database. The study assessed that CTNNB1 was significantly hypomethylated in LIHC samples as compared to normal control samples (**Figure 3**). As per studies gene promoter methylation level and gene expression have inverse relation^[40,41]. From this perspective, hypomethylation of CTNNB1 indicates upregulation in CTNNB1 expression and its role in the progression of LIHC.

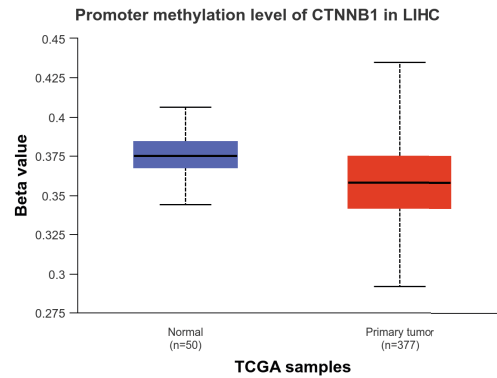


Figure 3. The promoter methylation level of CTNNB1 in LIHC and normal control samples using the UALCAN database.

3.4. Promoter methylation level of CTNNB1 in LIHC based on distinct characteristics

Subsequently, the study analyzed the promoter methylation level of CTNNB1 in LIHC based on different characteristics such as the patient's age, patient's gender, patient's race and pathological stages. Primarily, the study analyzed promoter methylation level of CTNNB1 in LIHC patient's pathological stages. The study evaluated that CTNNB1 was significantly hypomethylated in LIHC across individual cancer stages (**Figure 4A**). Next, the study assessed significant hypomethylation in CTNNB1 methylation level in LIHC based on the patient's race (**Figure 4B**). Eventually, the study examined that CTNNB1 was significantly hypomethylated in LIHC based on the patient's different age group and gender (**Figure 4C** and **Figure 4D**). This observation illuminates the hypomethylation of CTNNB1 across distinct characteristics, demonstrating its role in the progression of LIHC.

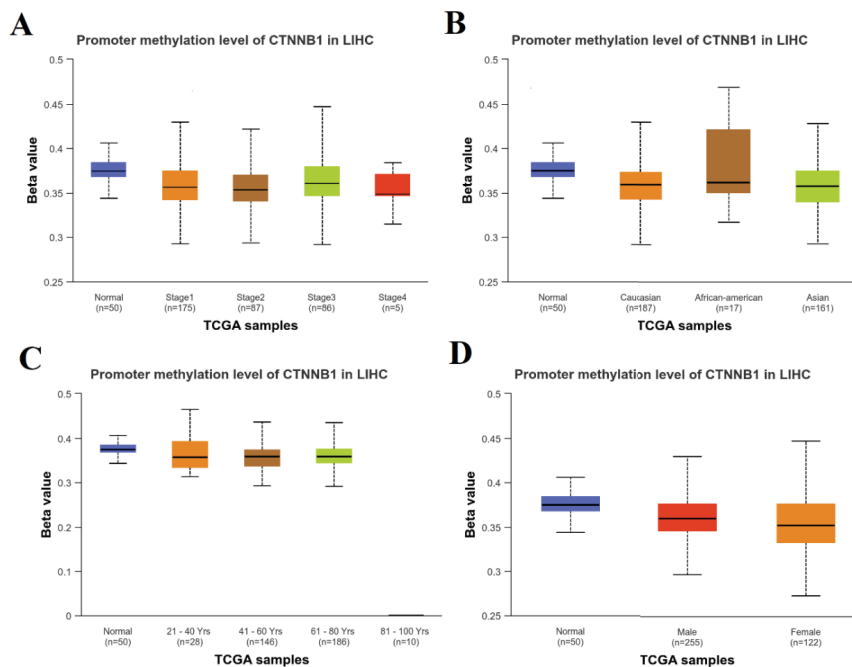


Figure 4. (A) Promoter methylation level of CTNNB1 in LIHC patient's individual cancer stage; (B) Promoter methylation level of CTNNB1 in LIHC patient's race; (C) Promoter methylation level of CTNNB1 in LIHC patient's age group; (D) Promoter methylation level of CTNNB1 in LIHC patient's gender.

3.5. Survival analysis of CTNNB1 in LIHC

The study performed a survival analysis employing the KM plotter to assess the role of CTNNB1 expression in the overall survival (OS) of LIHC patients. The study then investigated that overexpression of CTNNB1 leads to the worst OS, similarly, lower expression of CTNNB1 leads to the worst OS as indicated by significant *P*-value and higher hazard ratio. The calculated *P*-value is 0.045 which explains the statistically significant survival difference between the two groups. Based on this, the study assessed that overexpressed CTNNB1 in LIHC relates to a high mortality rate, illustrating its potential as a prognostic biomarker.

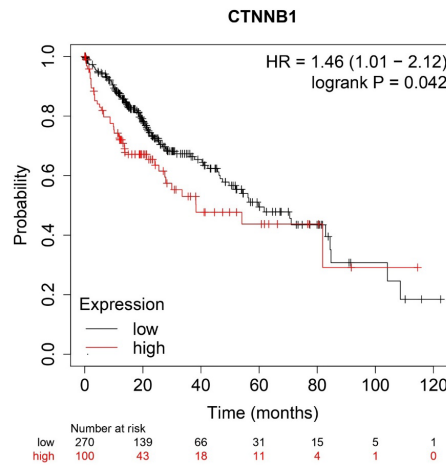


Figure 5. Survival analysis of CTNNB1 in LIHC using KM plotter.

3.6. Affirmation of CTNNB1 expression and survival analysis

The study utilized GEPIA2 to verify the detections of expression and survival analysis of CTNNB1. Firstly, the study analyzed CTNNB1 expression in LIHC and normal samples. Then, CTNNB1 was confirmed to be upregulated in the LIHC sample (**Figure 6A**), this result coincides with the previous investigation. Next, the study employed the box plot module to investigate CTNNB1 expression in LIHC pathological stages, and assessed variation but upregulation of CTNNB1 expression in various pathological stages (**Figure 6B**). This finding coincides with the previous observations, explaining that the upregulation of CTNNB1 expression leads to the progression of LIHC and has potential as a diagnostic biomarker.

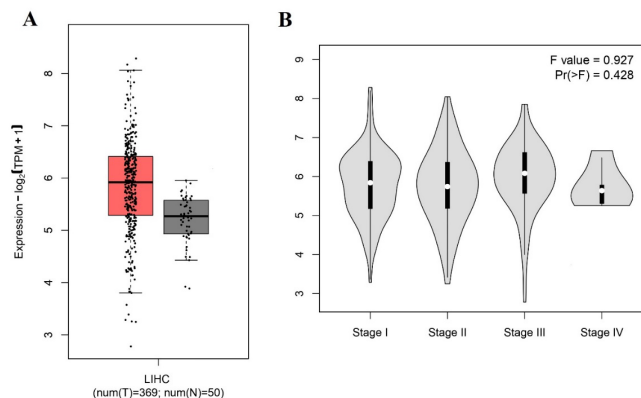


Figure 6. (A) Expression analysis of CTNNB1 in LIHC and normal control sample using GEPIA2; (B) Expression analysis of CTNNB1 in LIHC based on pathological stages using GEPIA2.

Following this, the study analyzed the impact of CTNNB1 expression on the OS of LIHC patients using the survival module of GEPIA2. The study analyzed that overexpressed CTNNB1 worst OS and lower expressed CTNNB1 had better OS (**Figure 7**). This result coincides with the previous findings, however, the difference is slightly significant because of P -value = 0.11. All of these findings suggest CTNNB1 overexpression has role in LIHC progression and with the worst OS of patients. This highlights the potential of CTNNB1 as diagnostic and prognostic biomarker.

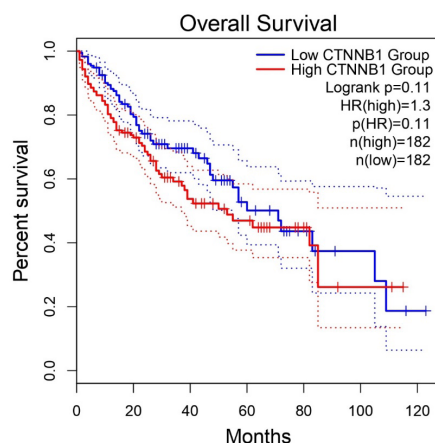


Figure 7. Survival analysis of CTNNB1 in LIHC using GEPIA2.

3.7. PPI network and pathway enrichment analysis of CTNNB1

In addition, the study widened the analysis by implicating protein-protein interaction (PPI) network construction, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The study expanded the analysis to evaluate the biological functions of CTNNB1. First, the study constructed a PPI network using the STRING database and unveiled interlinked 10 gens (**Figure 8**). This explains the diversity in the biological function of CTNNB1. Based on this, the study utilized the DAVID database and observed 6 terms for cellular component (CC), biological process (BP), molecular function (MF), and KEEG pathways by performing Go and KEEG analysis (**Table 1** and **Figure 9**). Then, the study performed KEEG pathway analysis and observed that genes were interlinked in several pathways with the Wnt signaling pathway, gastric cancer, Human papillomavirus infection, endometrial cancer and adherens junction pathways. These findings illustrate the role of CTNNB1 and associated genes in different processes.

Moreover, in GO analysis of CTBB1, the study analyzed substantial enrichment in biological processes such as proteasome-mediated ubiquitin-dependent protein catabolic process, negative regulation of canonical Wnt signaling pathway, positive regulation of transforming growth factor-beta.

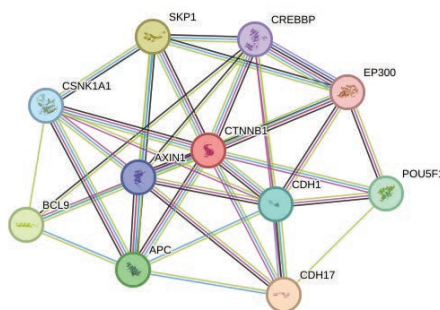


Figure 8. PPI network of CTNNB1 using STRING tool.

Receptor signaling pathway, canonical Wnt signaling pathway, N-terminal peptidyl-lysine acetylation, and macromolecular complex assembly (**Figure 9B**). Subsequently, in regard to cellular component, the study examines pivotal enrichment including beta-catenin destruction complex, catenin complex, lateral plasma membrane, Wnt signalosome, adherens junction, and transcription factor complex (**Figure 9C**). Furthermore, the study observed molecular function linked with CTNNB1 such as beta-catenin binding, RNA polymerase II sequence-specific DNA binding transcription factor binding, transcription coactivator activity, ubiquitin protein ligase binding, p53 binding, histone acetyltransferase activity (H3-K27 specific) (**Figure 9D**). These findings shed light on understanding the role of CTNNB1 and associated genes in different pathways and biological processes.

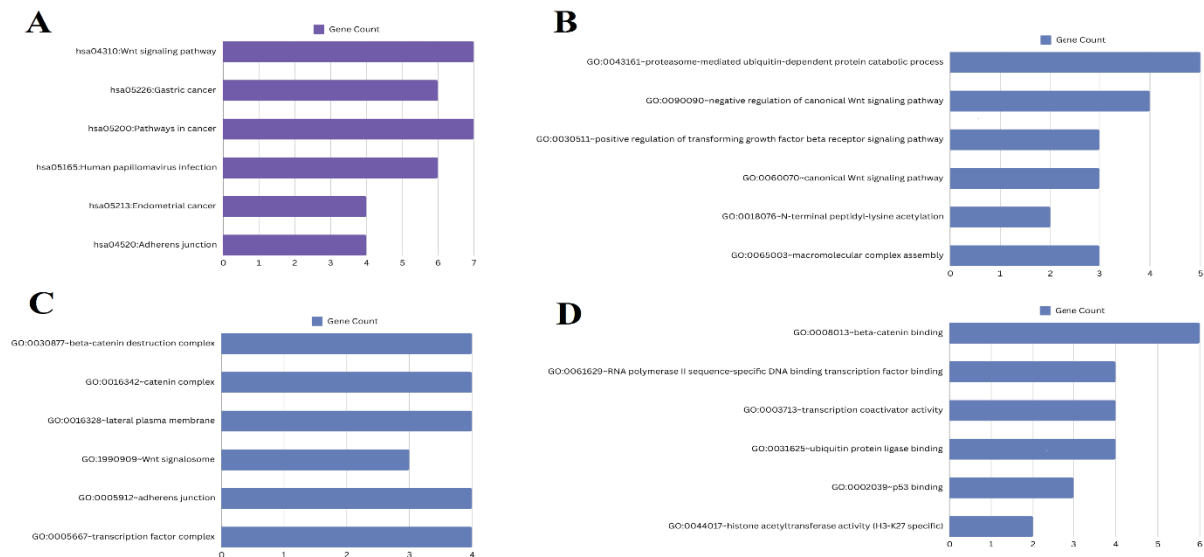


Figure 9. Kegg and GO analysis of CTNNB1 enriched genes utilizing the DAVID tool.

Table 1. Result of gene enrichment analysis

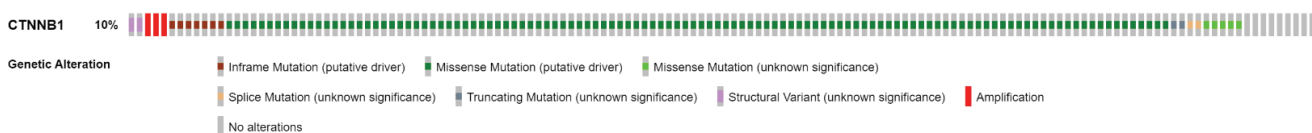
Gene term	Gene count	Genes	P-value
BP			
GO0043161: proteasome-mediated ubiquitin-dependent protein catabolic process	5	APC, CSNK1A1, AXIN1, CTNNB1, SKP1	3.736089222847483E-6
GO0090090: negative regulation of canonical Wnt signaling pathway	4	APC, CSNK1A1, AXIN1, CTNNB1	5.3413295934421694E-5
GO0030511: positive regulation of transforming growth factor beta receptor signaling pathway	3	CREBBP, AXIN1, EP300	1.3497253797827575E-4
GO0060070: canonical Wnt signaling pathway	3	BCL9, AXIN1, CTNNB1	0.0012638592484148154
GO0018076: N-terminal peptidyl-lysine acetylation	2	CREBBP, EP300	0.0015572278568399716
GO0065003: macromolecular complex assembly	3	CREBBP, APC, AXIN1	0.0023052682893945405
CC			
GO0030877: beta-catenin destruction complex	4	APC, CSNK1A1, AXIN1, CTNNB1	2.377141141438321E-8
GO0016342: catenin complex	4	APC, CDH1, CTNNB1, CDH17	4.102589855782143E-7
GO0016328: lateral plasma membrane	4	APC, CDH1, AXIN1, CTNNB1	8.05942485285713E-6

Table 1 (Continued)

Gene term	Gene count	Genes	P-value
GO1990909: Wnt signalosome	3	APC, AXIN1, CTNNB1	1.6623380306006684E-5
GO0005912: adherens junction	4	APC, CDH1, CTNNB1, CDH17	8.257006264142433E-5
GO0005667: transcription factor complex	4	CREBBP, EP300, CTNNB1, POU5F1	1.45523934603438E-4
MF			
GO0008013: beta-catenin binding	6	BCL9, APC, CDH1, AXIN1, EP300, SKP1	5.435587111301412E- 10
GO0061629: RNA polymerase II sequence-specific DNA binding transcription factor binding	4	CREBBP, EP300, CTNNB1, POU5F1	1.0554759656852471E-4
GO0003713: transcription coactivator activity	4	BCL9, CREBBP, EP300, CTNNB1	3.082509233862163E-4
GO0031625: ubiquitin protein ligase binding	4	APC, AXIN1, CTNNB1, POU5F1	4.918766119255133E-4
GO0002039: p53 binding	3	CREBBP, AXIN1, EP300	6.151526152403066E-4
GO0044017: histone acetyltransferase activity (H3-K27 specific)	2	CREBBP, EP300	0.001058901316322019
KEGG			
hsa04310: Wnt signaling pathway	7	CREBBP, APC, CSNK1A1, AXIN1, EP300, CTNNB1, SKP1	4.8186637813062545E-9
hsa05226: Gastric cancer	6	APC, CDH1, CSNK1A1, AXIN1, CTNNB1, CDH17	1.6789194931954612E-7
hsa05200: Pathways in cancer	7	CREBBP, APC, CDH1, AXIN1, EP300, CTNNB1, SKP1	3.6991021891065767E-6
hsa05165: Human papillomavirus infection	6	CREBBP, APC, CSNK1A1, AXIN1, EP300, CTNNB1	8.778542088270608E-6
hsa05213: Endometrial cancer	4	APC, CDH1, AXIN1, CTNNB1	2.3261089609916448E-5
hsa04520: Adherens junction	4	CREBBP, CDH1, EP300, CTNNB1	9.605435077106246E-5

3.8. Genetic mutation of CTNNB1 in LIHC

The study then investigated the genetic mutation of CTNNB1 and the role of this mutation in LIHC progression using cBioPortal. The analysis revealed a 10% mutation of CTNNB1 in LIHC. These observed mutations include in-frame mutation, missense mutation, splice mutation, truncating mutations, structural variant and amplification (**Figure 10**). These findings suggest that genetic mutation of CTNNB1 has a significant role in the proliferation of LIHC. That explains the potential of CTNNB1 as a therapeutic biomarker in LIHC.

**Figure 10.** Genetic mutation of CTNNB1 in LIHC.

4. Discussion

Cancer is a fatal disease that has been in focus of thorough scientific investigation for decades ^[42]. In this disease, uncontrolled cell division results in various types of malignancies ^[43]. While liver cancer is the 5th most common cancer with 2nd most mortalities worldwide, 90% of liver cancers are liver hepatocellular carcinoma (LIHC) ^[1]. LIHC is mostly diagnosed at advance stages, it shows resistance to chemotherapy, target therapy and therapeutic strategies. Therefore, there is an urgent need to discover biomarkers. This study used bioinformatics tools to evaluate CTNNB1's potential as a diagnostic, prognostic and therapeutic biomarker in LIHC. CTNNB1 is a gene that is located on human chromosome 3p21–22, it regulates various signaling pathways such as Wnt/ β -catenin and Hippo pathway. CTNNB1 mutation is associated with the progression of different cancers ^[44]. CTNNB1 variation disturbs the signaling pathways which leads to the progression of LIHC and other cancers. This study performed expression analysis, survival analysis, gene enrichment analysis and gene mutation of CTNNB1 in LIHC using bioinformatics tools.

The study utilized the UALCAN database to analyze CTNNB1 expression in LIHC and evaluated that CTNNB1 was significantly overexpressed in LIHC as compared to normal samples. Moreover, this study analyzed CTNNB1 expression based on various variables like individual cancer stage, patient's age, gender and patient's race, unveiling significant overexpression. All of these findings proposed that CTNNB1 has a role in the progression and development of LIHC.

In addition, this study analyzed promoter methylation levels of CTNNB1 in LIHC UALCAN. The study analyzed hypomethylation in the CTNNB1 promoter region in LIHC as compared to the normal control sample. This hypomethylation of CTNNB1 supports upregulation in expression, as previously it's been stated that genes promoter methylation level and expression have an inverse relation ^[45]. Further, hypomethylation of CTNNB1 was investigated in LIHC when analyzed based on different parameters such as LIHC pathological stages, patient age, gender and race. These findings acknowledge previous analysis that hypomethylation regulates the upregulation of CTNNB1 expression and plays a role in the progression of LIHC.

Moreover, the study performed a survival analysis of CTNNB1 in LIHC by employing a KM plotter, which revealed that overexpression is associated with the worst OS of LIHC patients. The study checked the validation of the result by utilizing GEPIA2 to perform expression and survival analysis. This study investigated that CTNNB1 was overexpressed in LIHC in contrast with normal samples. Expression analysis in LIHC pathological stages using GEPIA2 was also supports upregulation of CTNNB1. Moreover, survival analysis with GEPIA2 revealed that overexpression is linked with worst OS and lower expression is linked with better OS. But, the difference between these groups is not statistically significant. These similarities in results proposed that CTNNB1 have role in development and progression of LIHC.

A group of ten genes directly linked with CTNNB1 was analyzed in protein-protein interaction (PPI) network analysis. While in pathway enrichment analysis, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed. KEGG analysis identified enriched pathways associated with CTNNB1 and linked genes such as the Wnt signaling pathway, gastric cancer, Human papillomavirus infection, endometrial cancer and adherens junction. GO analysis revealed pathways specially enriched linked with CTNNB1 including negative regulation of canonical Wnt signaling pathway, positive regulation of transforming growth factor beta receptor signaling pathway, canonical Wnt signaling pathway, beta-catenin destruction complex, catenin complex, lateral plasma membrane, Wnt signalosome, adherens junction, transcription factor complex, beta-catenin binding, RNA polymerase II sequence-specific DNA binding transcription factor binding,

transcription coactivator activity, ubiquitin protein ligase binding, p53 binding and histone acetyltransferase activity (H3-K27 specific). These pathways accounts for especially negative regulation of canonical Wnt signaling pathway, p53 binding, beta-catenin destruction complex and such others are recognized for their role in LIHC progression.

While cBioPortal revealed 10% of genetic alteration of CTNNB1 in LIHC. These observed mutations include in-frame mutation, missense mutation, splice mutation, truncating mutations, structural variant and amplification. These results suggest that CTNNB1 mutation has strong role in the progression of LIHC. Comprehensively, these findings suggest that CTNNB1 has potential as a diagnostic, prognostic and therapeutic biomarker in LIHC.

5. Conclusion

In overview, our detailed examination of CTNNB1 expression, prognostic significance, and genetic mutations in LIHC utilizing UALCAN, GEPIA2, KM plotter, and cBioPortal uncovered significant understanding. This study discovered a correlation between higher CTNNB1 expression levels and the progression of LIHC. These findings illuminate CTNNB1's potential as a diagnostic, prognostic, and therapeutic biomarker in LIHC, emphasizing its pivotal role in disease management.

Disclosure statement

The authors declare no conflict of interest.

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Case Report: B-cell Acute Lymphoblastic Leukemia/ Lymphoma Following Castleman Disease

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Abstract: Castleman disease (CD) is a rare nonmalignant lymphoproliferative disorder presenting systemic symptoms such as fever, night sweats, fatigue, anemia, effusions, and multifocal lymphadenopathy. The etiology of CD has not been clarified to date. The coexistence of CD with B-cell acute lymphoblastic leukemia/lymphoma (B-ALL/LBL) has been rarely reported. Although the pathogenesis remains unclear, this association probably reflects an incidental and fortuitous finding rather than the alteration of a common pluripotent stem cell precursor. Herein, the study reports on one case of CD coexisting with B-ALL/LBL and elucidates the underlying mechanism of pathology in some aspects.

Keywords: Castleman disease; B-cell acute lymphoblastic leukemia; Case report

Online publication: Jan 1, 2025

1. Introduction

Castleman disease (CD) is a rare lymphoproliferative disorder first described 60 years ago by Dr. Benjamin Castleman ^[1]. It is a group of clinicopathologic disorders with similarities in histopathology and clinical features found in hematology, oncology, rheumatology, and virology ^[2]. A large number of case reports and reviews have examined the clinical manifestations ^[3], pathologic features ^[4], and treatment ^[5] of this complicated condition in recent decades. One of the key characteristics of CD is its heterogeneous nature, making diagnosis and treatment challenging. It can present as either a localized or systemic disease, and its symptoms can range from mild to severe. CD is also known to be linked to other underlying medical conditions, such as human immunodeficiency

virus (HIV) infection, and certain forms of cancer. Treatment options for CD depend on the specific form and severity of the disease and may involve the use of immunosuppressive drugs, biological agents, and in some cases, surgical intervention ^[6]. The management of CD often involves a multidisciplinary approach, with collaboration between specialists in different fields to provide the best possible care for patients ^[7-9].

B-cell acute lymphoblastic leukemia/lymphoma (B-ALL/LBL) is a common type of blood cancer ^[10,11]. It is primarily a childhood disease, but relapsed pediatric and adult patients have poor prognoses ^[12]. It is characterized by the rapid overproduction of malignant immature hematopoietic cells that inhibit normal hematopoiesis in the bone marrow and invade peripheral organs ^[13,14]. Studies have shown that CD patients have a significantly higher risk of severe allogeneic cytopenia, multi-organ failure, and lymphoma ^[15]. There are still 10% of patients who fail to respond to initial treatment and 40% to 70% who relapse despite improvements in treatment over the past 30 years ^[16].

It is quite a rare event for CD to progress to B-ALL/LBL, which belongs to different pathogenic cell types. This case report first reports a case of a CD patient who progressed to B-ALL/LBL and was treated with thalidomide and retinoic acid, respectively.

2. Case report

2.1. Case description

The patient, a female, presented with abdominal distension, loss of appetite, general weakness, and palpitations in 2002 with no obvious cause. She was treated for hepatitis and gastritis at local hospitals (Tianchang Hospital, Anhui Province, and Tiankang Hospital, Tianchang City, Anhui Province) without obvious abnormalities. Blood pressure was 160/100 mmHg, the abdominal circumference was 83 cm, blood tests were normal, and an MRI image of the upper abdomen showed “enlarged liver and spleen with signs of ascites in the abdominal cavity; MRV of the upper abdomen showed that the inferior vena cava was slightly distorted and flattened in the hepatoportal region, suggesting cirrhosis with massive ascites.” The diagnosis of Buga syndrome was considered, and he was admitted to Jiangsu Provincial People’s Hospital, where he was proposed to undergo vascular dissection. The lymph node biopsy was performed because of the enlarged lymph nodes in the neck found 2 d before the operation, and the pathology on April 6, 2004, showed that the vascular giant follicular lymphoid tissue was hyperplastic, further immunotyping confirmed the diagnosis of CD. 2 courses of chemotherapy were given to the CHOP regimen, and the lymph nodes subsided, and the patient was transferred back to Tiankang Hospital for further chemotherapy. After the 3rd course of CHOP, the patient was discharged from the hospital on August 1st, 2004, and switched to immunotherapy: alpha-interferon (300 U subcutaneous injection) twice a week for 6 months. However, the patient’s condition was unstable during immunotherapy, and ascites persisted and worsened from time to time. 6 months later, following 7 courses of the CHOP regimen, hypoproteinemia developed. At the same time, human albumin was given for long-term maintenance (20 g each time), but the results were poor, and ascites still existed as well as a drug reaction - peripheral neuritis. The above conditions made the treatment difficult to carry out.

On March 25, 2006, this patient was treated with a trial of thalidomide (no CHOP-related treatment). After one month of treatment, the patient’s ascites had significantly decreased, the spleen and liver had significantly decreased in size, and the patient’s condition had improved steadily. During thalidomide administration, the patient complained of weakness, loss of appetite, and occasional manifestations of peripheral neuritis, but they were relatively mild, and no special treatment was given. The patient’s cumulative chemotherapy drug dosage: total immunosuppressant (CTX) 7.4 g, total vincristine (VCR) 14.0 mg, total vincristine 90 mg, total human albumin 3000 g. On March 25, 2006, he was given 300 mg daily (100 mg tid) of Response Stop. Along 47 months, total

dose of Reactive Discontinuation: 391,500 mg. (After stabilization of the disease, the patient missed and refused doses due to side effects associated with thalidomide: malaise, peripheral neuritis, and constipation, which decreased after education).

Table 1. Patient treatment time points

Date	Patient's condition
Early 2002	Onset of disease
2004	CHOP-based regimen 10 times
March 25, 2006	Thalidomide 300 mg/d
July 2009	Thalidomide dosage reduced to 150 mg/d
February 2010	Discontinue thalidomide

After 7 years of discontinuation, on April 22, 2017, the patient had recurrent leukopenia, during which he was treated with recombinant human granulocyte-stimulating factor injection for leukocyte elevation. On February 6th, 2023, the patient was diagnosed with B-ALL/LBL by bone marrow cytology (2.3), bone marrow biopsy (2.4), immunohistochemical markers, immunophenotyping (2.5), and pathology (2.6). Patients were treated using GM-CSF+All-trans retinoic acid (ATRA) with more favorable results. In addition, after treatment with translational medicine, the patient's condition improved and persisted for 2 two years.

Table 2. Changes in patients' leukocyte concentrations during treatment

Test date	Leukocyte concentration (*10 ⁹ /L)	Main diagnosis	Main treatment methods
September 24, 2017	3.00	Leukopenia + CD	Thalidomide
April 25, 2018	3.10		
October 15, 2018	2.00		
March 13, 2019	2.00		
December 06, 2020	3.24		
December 08, 2020	2.27		
December 10, 2020	1.82		
December 16, 2020	2.33		
December 17, 2020	1.79		
December 19, 2020	2.93		
December 21, 2020	5.20		
December 25, 2020	1.75		
December 29, 2020	1.79		
January 02, 2021	1.15		
February 01, 2021	2.73		
February 02, 2021	10.07	B-ALL/LBL	GM-CSF+All-trans retinoic acid (ATRA)
February 05, 2021	8.42		
September 18, 2022	3.56		

2.2. Bone marrow cytology

The patient underwent bone marrow cytology (**Figure 1**). On December 14, 2020, the results showed active nucleated cell proliferation and detected a class of morphologically similar atypical lymphocytes (nature to be determined) in 32.5% of the cases as well as altered granulomatous morphology. On May 3, 2021, and on October 8 of the same year, bone marrow cytology results indicated active nucleated cell proliferation and occasional atypical lymphocytes. However, On September 21, 2022, bone marrow cytology results indicated that nucleated cell proliferation was markedly active, and abnormal cells were detected in approximately 60% of cases.

On October 2, 2022, the patient underwent bone marrow histopathology (**Figure 2**) and the diagnosis was that the patient had characteristics of leukemia. Hematopoietic tissue hyperplasia is active, with a volume of about 50–60%, and adipose tissue hyperplasia is reduced. Granular, red, megakaryocyte proliferation is reduced, rare; naïve cell proliferation, medium cytosol, cytoplasm is less, the nucleus is round or slightly irregular, diffuse or focal distribution; a little fibrous tissue proliferation is seen scattered.

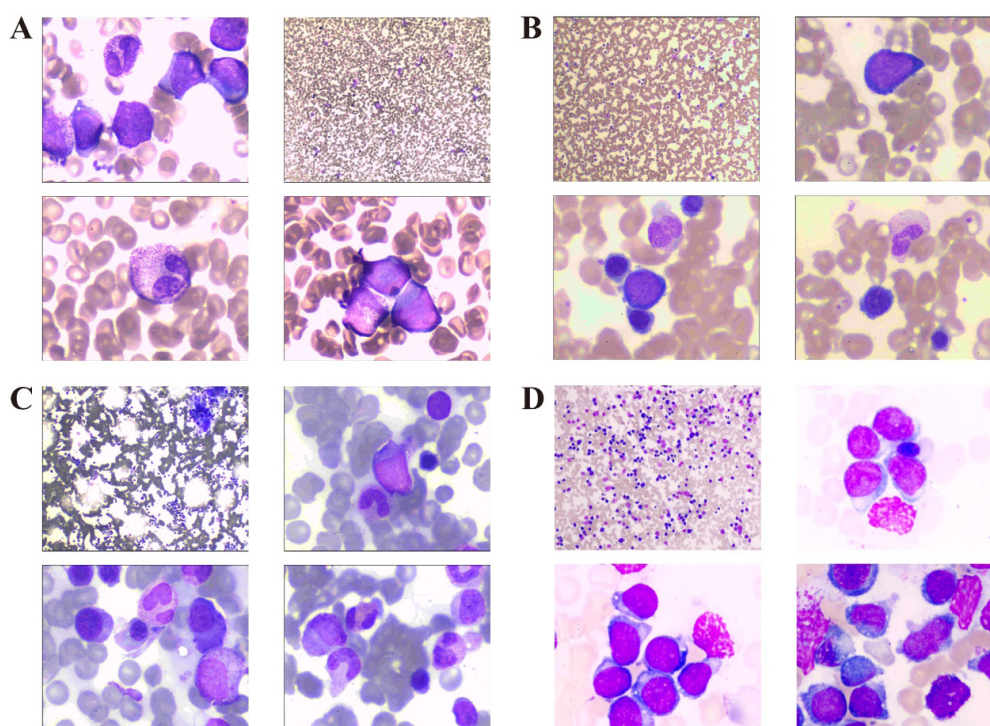


Figure 1. Bone marrow cytology results on different dates. (A) December 14, 2020; (B) May 3, 2021; (C) October 8, 2021; (D) September 21.

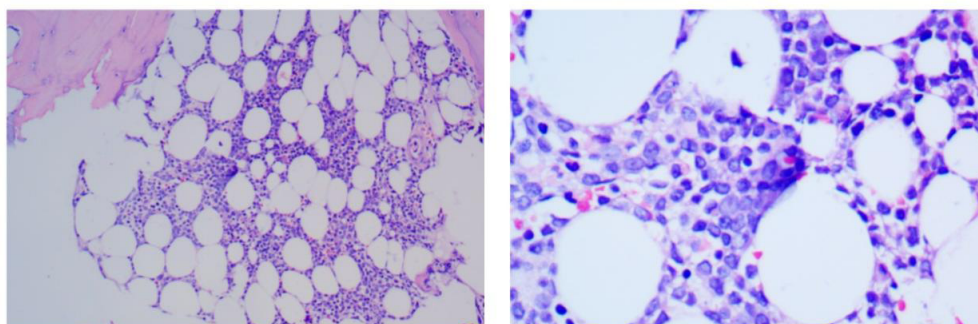


Figure 2. Bone marrow biopsy.

2.3. Immunophenotyping

On October 4, 2022, the patient was immunophenotyped by flow cytometry (**Figure 3**). Gate analysis was set up on a CD45/SSC dot plot, and a population of abnormal cells was visible in the distribution area of the primitive extension towards CD45-negative, not demarcated from nucleated erythrocytes, totaling approximately 83% of the nucleated cells, which positively expressed HLA-DR, CD10, CD19 (dim), CD33, CD34, CD58, CD123, cCD79a, and TdT. Myeloid proliferation was markedly suppressed. The results showed that the patient was compatible with the immunophenotype of B-ALL/LBL. On February 8, 2023, the patient was again immunophenotyped by flow cytometry and the diagnosis remained B-ALL (**Figure 4**).

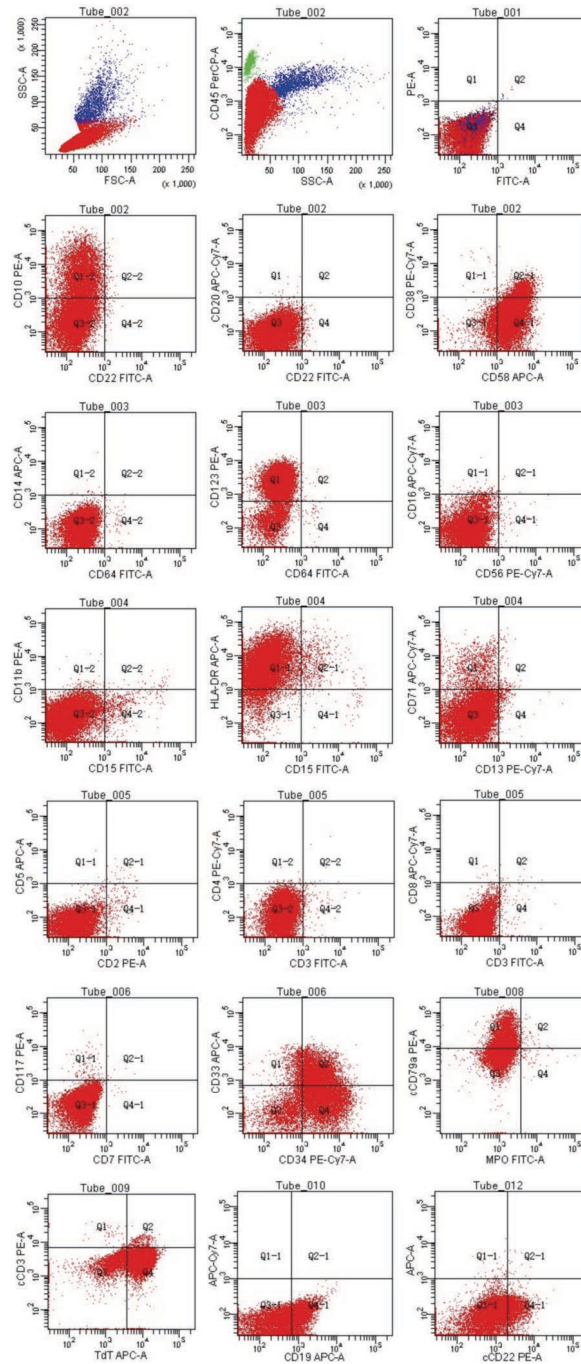


Figure 3. Immunophenotyping (October 4, 2022).

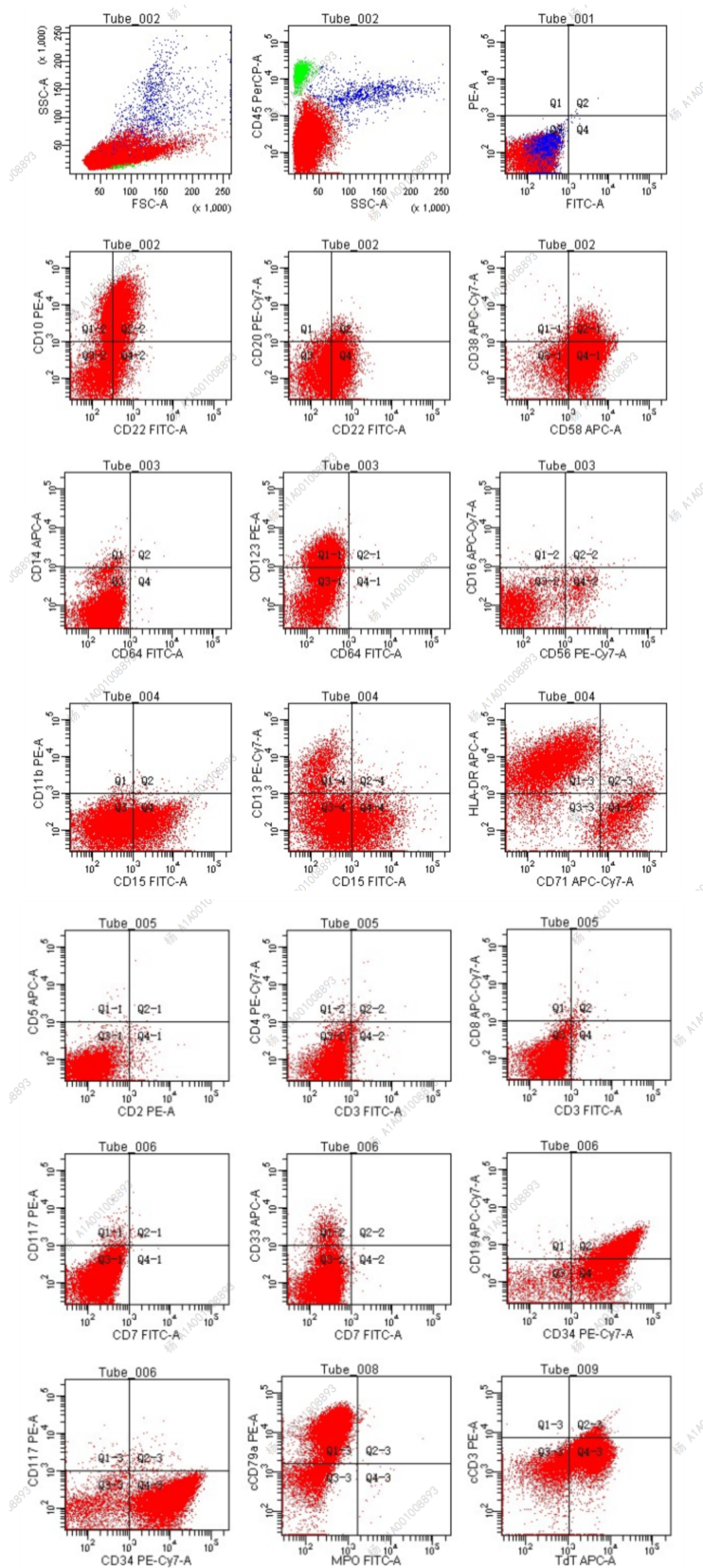


Figure 4. Immunophenotyping (February 8, 2023).

2.4. Pathology

On February 6, 2023, the patient underwent a bone marrow biopsy for pathology (**Figure 5**). The results showed that the patient had a tumor of the lymphohematopoietic system, which was considered to be B-ALL.

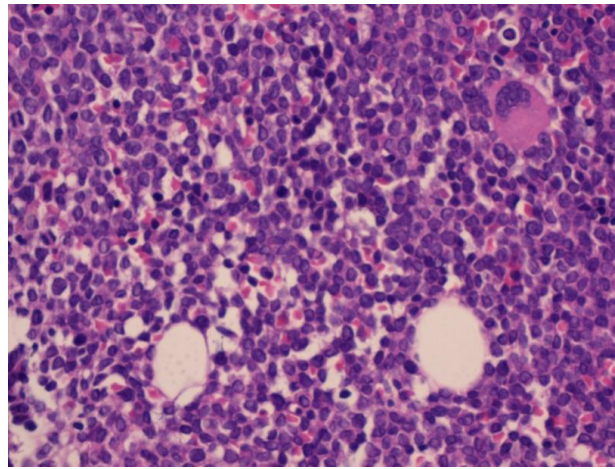


Figure 5. Bone marrow biopsy for pathology.

3. Discussion

The pathogenesis of CD is unknown and can affect the growth of B lymphocytes across multiple medical disciplines, including hematology, oncology, rheumatology, and virology. Patients with CD may present with symptoms such as fever, night sweats, fatigue, anemia, and lymphadenopathy, which are caused by elevated levels of IL-6 and other pro-inflammatory cytokines. Three main therapeutic approaches have been used to manage CD, including anti-inflammatory and immunosuppressive therapies, elimination of cytotoxic treatments that cause hypercellularemia, and blocking of IL-6 signaling with mAbs ^[17]. Glucocorticoids are also commonly used, but their effects are often limited and temporary, with symptoms frequently reappearing as the dose is reduced. In severe cases, autologous stem cell transplantation has shown good results ^[18]. Recent studies have shown that multicentricity, histopathological type, and anemia are important risk factors for reducing progression-free survival ^[19]. In 2004, there was no established treatment protocol for CD, and treatment was performed concerning that of lymphoma, with a brief period of stabilization after CHOP and add-on therapy, followed by persistent and progressive decreasing hypoproteinemia, and thoracoabdominal fluid.

Thalidomide is an effective immunomodulator that inhibits the production of a variety of cytokines, including IL1, IL6, IL12, tumor necrosis factor- α , and VEGF. It has been shown that thalidomide is effective in relieving CD ^[20–22]. Meanwhile, in the year 2004, considering that there was no readily available reference treatment protocol here, after full communication with the family, the study used thalidomide for treatment. After the use of thalidomide, the dose of albumin was reduced, and after half a month, ascites were reduced, and for about a month ascites disappeared, and the treatment achieved good results. There is no treatment-related side effects occurred, and the patient showed sustained clinical improvement.

Autoimmune diseases are a recognized risk factor for malignant lymphoma. It has been shown that autoimmune diseases such as dry syndrome, systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroiditis, celiac disease, and herpes-like dermatitis are associated with an increased risk of malignant lymphoma ^[23–24]. However,

the exact mechanism has not been clarified and may be due to immune dysregulation with abnormal immune responses and reduced tumor immune surveillance. It has been suggested that CD has an intrinsic tendency to progress to lymphoma, but the molecular mechanisms involved in its transformation remain unclear ^[25]. The autoimmune manifestations of CD patients and the discovery of auto-reactive T cells and pathogenic auto-antibodies would support its being an autoimmune disease ^[26]. From these results, Castleman's disease is likely the underlying disease that triggers B-ALL. The association between it and B-ALL/LBL is complex and the relationship between CD and macrophages is still unknown. Several studies have speculated that Castleman disease is likely to be the disease underlying the immune factors that trigger B-ALL. Through the lens of translational medicine, the patient transformed from CD to B-ALL. After patients were diagnosed with B-ALL, the study first treated them based on the CHOP regimen, however, the results were not satisfactory. At the same time, the high cost of CHOP-based treatment options makes it difficult for patients' families to afford the high cost of treatment. After full communication with the patient's family and also in conjunction with some cases of translational medicine ^[27], the treatment plan was switched.

Macrophages are one of the important effector cells that perform immunosurveillance functions in the body and can kill tumor cells extracellularly by secreting and releasing soluble cytotoxic factors. Macrophages are highly versatile and heterogeneous cells that play a key role in both innate and adaptive immunity. In response to environmental stimuli, macrophages can differentiate into two subpopulations, classical (M1) or alternative (M2) activated macrophages. M1 polarization is characterized by the ability of macrophages to produce high levels of pro-inflammatory cytokines, increased expression of co-stimulatory molecules, and increased efficiency of antigen presentation, which supports its function of clearing tumor cells. In contrast, M2 macrophages mediate immunomodulatory functions through the production of anti-inflammatory cytokines and higher levels of scavenger mannose receptors and mainly exhibit immunosuppressive properties ^[28–30]. On the one hand, the M1/M2 polarization state can disrupt the balance of lymphocyte subpopulation differentiation or alter their tolerogenic clearance ^[21–33], leading to the pathogenesis of autoimmune diseases ^[34–36], and immune defects of genetic, iatrogenic or infectious origin may play an important role in CD development ^[37]. Burren *et al.* (2016) found that Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with CD, while Bhaskaran *et al.* (2017) found that KSHV infection induces M2 polarization ^[38,39], which suggested M2 polarization may contribute to the occurrence and development of CD. On the other hand, accumulating evidence suggests that M2 polarization may contribute to a variety of cancers, including lung cancer, pancreatic cancer, breast cancer, etc. and may promote cancer metastasis ^[40–42]. In addition, *in vitro* experiments have also confirmed that promoting M1 polarization can induce B-ALL cells apoptosis ^[43]. Although there is currently a lack of convincing *in vivo* experiments and the role of tumor microenvironment (TME) in B-ALL is still poorly understood, the above research can still make polarization become a potential research direction for the connection between CD and B-ALL.

M1 macrophages exhibit good anti-tumor properties by antigen presentation to T cell receptors and recruiting CD8⁺T and NK cells to the TME, while M2 macrophages are associated with poor prognosis in many tumors. In the early stages of tumors, due to the chronic inflammatory environment in TME, the proportion of M1 macrophages is relatively high. But as tumor cells secrete a large amount of M2-like cytokines (such as IL-10, CCL-2/3/4/5/7/8, CXCL12, VEGF, etc.), M2 macrophages begin to rapidly increase ^[44]. Although most M2 macrophages can secrete anti-inflammatory cytokines such as IL-10, but to its ability to divert cytotoxic T cells to kill malignant cells, the pro-inflammatory nature of the TME has not impacted ^[45]. However, when the transformation between M1 and M2 phenotypes occurs whether macrophages have an antitumoral ability to

eradicate aberrant malignant cells before the formation of tumor still remains unclear^[46]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an autocrine and a paracrine cytokine. It stimulates the growth, differentiation, and function of normal and leukemic myeloid progenitors, which activate immune system cells^[47-49]. GM-CSF augments both innate and adaptive immunity by facilitating the growth and function of neutrophils, macrophages, monocytes, and dendritic cells^[50,51]. GM-CSF can induce macrophage polarization toward M1. GM-CSF has also been implicated in leukemogenesis, although altered regulation of GM-CSF expression in myeloproliferative disorders is complex. In this case, significant efficacy was observed by using GM-CSF to increase leukocyte levels.

In the recent years, many malignant tumors are associated with autoimmune diseases. This is usually believed to be related to chronic inflammation caused by autoimmune diseases^[52]. Chronic inflammation can lead to tissue and cell damage, accompanied by a certain degree of repair and fibrosis. In this cycle of destruction and regeneration, the stability of genes is disrupted, ultimately leading to malignant cell mutations. Subsequently, immune cells will recruit to malignant cells and lead to new inflammation, further leading to tumor invasion and metastasis. All-trans retinoic acid (ATRA) is a bioactive derivative of vitamin A. In general, it promotes cell maturation differentiation and apoptosis by binding to specific nuclear receptors and can be mediated by non-genomic signal pathways, such as MAPK and PKA^[53]. It can reverse certain pre-cancerous cells and even cancerous cells into normal cells^[54]. It has been successfully used in precancerous lesions and cutaneous T-cell lymphomas. Acute promyelocytic leukemia, promotes the maturation of primitive cells, thereby alleviating the severe bleeding associated with the disease. Hung et al. (2008) found that ATRA could exert its anti-inflammatory effects by inhibiting the production of macrophage inflammatory cytokines such as JNK-AP-1 signaling pathway expression minus iNOS, NO, and COX-2, thereby protecting against LPS-induced organismal injury^[55]. Zhang *et al.* (2019) found that ATRA could also inhibit LPS-stimulated macrophage pro-inflammatory factors IL-1 β , TNF- α , and iNOS production through direct activation of protein kinase, TNF- α , and iNOS production^[56]. Moreover, there have been many reports regarding the cell biological effects of ATRA on human myeloma cells and a few clinical trials. Most of these reports have revealed growth inhibition by ATRA mediated by down-regulation of the IL-6/IL-6R auto/paracrine loop and upregulation of p21/Cip1^[57]. However, He *et al.* (2022) found that ATRA can promote M2 polarization of macrophages in an inflammatory environment^[58], so it was combined with GM-CSF to enhance its therapeutic effect.

Studies have shown a synergistic effect between ATRA and GM-CSF^[59]. Treatment of human myeloblastic leukemia with ATRA in combination with GM-CSF enhances granulocytic differentiation. The study used GM-CSF to increase leukocyte levels in patients with significant efficacy. First, recent study showed that during *in vitro* ATRA and GM-CSF treatment of healthy bone marrow progenitor cells, stimulation of normal granulocytes was observed^[60], possibly due to increased sensitivity to growth factors. Secondly, in bone marrow tissue cultures of patients with chronic granulocytic leukemia, ATRA inhibits the clonal expansion of stem cells. In the present case, the patient was in ineffective remission with GM-CSF and ATRA. The relapse of the disease in the middle of the process may be related to the infection with COVID-19, which made the treatment disturbed.

In addition to GM-CSF, in the subsequent analysis and reference of the case, it was found that IL-2, another immunostimulant, may also play its biological functions by inducing polarization^[61] and its γ chain subunit can be well combined with FERM domains of JAK1 and JAK3, which is a hot spot for hematologic malignancies^[62]. Besides, it was also found that IL-2 exerts its therapeutic value in other types of tumors by activating, stimulating the proliferation and affecting the phenotype of other immune cells, such as NK cells, DC cells and T cells^[63-69].

However, related studies are mainly animal or cell experiments. Due to the species specificity of biological therapeutic agents, more studies are needed to verify their clinical value. Therefore, future research can also explore its therapeutic value in similar diseases.

To summarize the present paper, a few highlights will be listed:

- (1) CD is a benign and rare lymphoproliferative disease;
- (2) Treatment of CD with thalidomide is first reported in China;
- (3) Long-term, stable control of CD with thalidomide treatment;
- (4) CD can turn into B-ALL/LBL;
- (5) Treatment of B-ALL/LBL by translational medicine protocols through ATRA and GM-CSF.

4. Conclusion

In conclusion, the study reports a rare case of CD that was later found to have B-ALL/LBL, and it treated CD with thalidomide and B-ALL/LBL with retinoic acid. Future studies can not only confirm the hypothesis proposed in this case through *in vivo* and *in vitro* experiments, but also focus on the relationship between CD and macrophage polarization and the dynamic polarization of macrophages in TME. In addition, the target molecules affecting macrophage function in the disease can be analyzed by bioinformatics methods to further explore the detailed molecular mechanism of the disease and its treatment strategies.

Authors' contributions

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Disclosure statement

The authors declare no conflict of interest.

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Electrospinning - A Potential Bio-Fabrication Method for Developing Various Tissue Engineering Scaffolds

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Abstract: Scaffolds in tissue engineering provide essential support for new tissue growth. Such scaffolds could be fabricated from materials like natural and synthetic polymers with prime properties such as biocompatibility and mechanical strength. Among other developments made, electrospinning has been a significant factor in making intricate scaffolds that imitate the extracellular matrix of tissue. It gives various properties in the fibers for specific applications by the controlled parameter conditions like voltage and flow rate. It is innovations like multi-component fibers and 3D structures that assist in the problem of uniformity and mechanical strength. Electrospinning research still is on the front line in increasing its potential applications in tissue engineering, filtration, and drug delivery. Process parameters optimization is among the strategies deployed to lessen the electrospinning problem of bending instabilities. The modified setups offer fiber production versatility. The setups introduced include far-field electrospinning that provides long, directed nanofibers and near-field electrospinning that gives good fiber deposition. Electromechanical spinning unifies electrical and mechanical aspects to have controlled fiber properties. In the area of applications of electrospun nanofibers, so far, the areas like biomedical, environmental, energy, textile, sensor, agriculture, cosmetic, and food packaging industries come as a real versatile bunch. This potential of the technology in divergent fields is ever-growing, in ongoing research continues to enhance its effectiveness toward tissue engineering solutions.

Keywords: Scaffolds; Electrospinning; Tayler's Cone; Bending instabilities; Application of electrospinning

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1. Introduction

Tissue engineering scaffolds are structures that provide an enabling environment where cells can grow and increase to finally develop into a functional tissue or organ in a three-dimensional way^[1]. Therefore, such scaffolds should replicate as closely as possible the natural extracellular matrix within the body tissues to instruct cellular

behavior and tissue formation. They can be prepared from a variety of materials, either natural polymers like collagen or fibrin, synthetic polymers such as polycaprolactone or poly(lactic-co-glycolic acid), or a combination ^[2]. An ideal tissue engineering scaffold should exude biocompatibility and biodegradability, be mechanically strong and porous, and facilitate cell adhesion, proliferation, and differentiation. Besides, the scaffolds should have the proper pore size and interlinked networks to facilitate the diffusion process of the required nutrients and oxygen, removal of waste, and growth of tissues. In addition, scaffolds can be modified to have physicochemical properties that meet specific tissue or organ requirements by modulating composition, structure, and mechanical properties. Such innovations in scaffold design and manufacturing methods, such as 3D printing and electrospinning, have resulted in the realization of more intricate and functional scaffolds for several tissue engineering purposes. Tissue engineering scaffolds are three-dimensional designs for supporting the growth of new tissue. These scaffolds act as a provisional matrix on which the cells attach themselves, start proliferating, and eventually form new tissue that aids in restoring parts of the body that may be damaged ^[3].

Scaffolds in tissue engineering are designed using various materials ^[4,5], including natural polymers like collagen and hyaluronic acid, synthetic polymers such as polylactic acid and polycaprolactone, and composite materials that combine the strengths of both. Essential properties of scaffolds include biocompatibility to support cell growth without immune reactions, biodegradability to synchronize with tissue formation without leaving toxic residues, mechanical strength to bear loads until tissue regeneration, and high porosity for cell migration and nutrient exchange. Common fabrication techniques include electrospinning for creating fibrous, porous structures, 3D printing for precise architectural control, freeze-drying to produce porous scaffolds, and solvent casting with particulate leaching to form porous structures ^[2,6].

Tissue engineering applications span various fields, including bone, cartilage, skin, cardiac, and nerve tissue engineering, each leveraging scaffolds for cell growth, repair, or regeneration ^[7,8]. Scaffolds also address critical challenges like ensuring vascularization, minimizing immune responses, and achieving functional integration of new tissues with existing ones. Ongoing research focuses on improving scaffold materials, fabrication methods, and strategies for effective tissue integration to enhance the efficacy and applicability of tissue engineering solutions.

2. Electrospinning

Electrospinning is one of the newest and most versatile techniques used in the fields of nanotechnology and materials science. In this process, ultrafine fibers are created by applying an electric field to a polymer solution or melt ^[9]. The resulting fibers may have diameters in the range between a few nanometers and several micrometers. Main features of the electrospun fibers are a high surface area to volume ratio, porosity, and high aspect ratio. These features make it possible to use electrospun fibers in such areas of technology as tissue engineering, drug delivery, sensors, filtration, protective clothes, and energy storage devices. The electrospinning process has a great deal of tunability, with the possibility of changing parameters such as polymer concentration, solvent type, applied voltage, and the distance between the spinneret and collector to control fiber diameter, alignment, and morphology. Such precision allows the tailoring of fiber properties for a given application. In short, electrospinning represents a new powerful technology that drives innovations in every walk of life, acting as the seat of material innovation for new materials with very unique properties as well as their applications. These will be continued as an important field of development in tissue engineering scaffolds, where much active research is now focused on overcoming

current challenges and extending its applications ^[10–15].

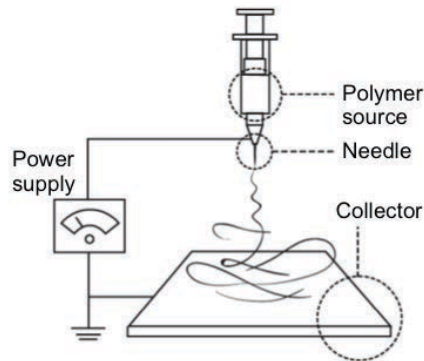


Figure 1. Experimental setup for electrospinning.

2.1. Electrospinning experimental setup

Electrospinning produces nanofibers by applying a high voltage to a polymer solution. The experimental setup normally consists of a few key components:

- (1) High voltage power supply: It provides the appropriate voltage needed to generate an electric field between the spinneret and collector.
- (2) Spinneret: This may be a needle or nozzle through which the polymer solution is extruded. The nanofiber diameter would depend on the diameter of the spinneret.
- (3) Syringe pump: This sets the flow rate of the polymer solution through the spinneret.
- (4) Collector: Grounded plate or drum upon which the nanofibers collect.
- (5) Polymer solution reservoir: Container to hold polymer solution, feeding into the syringe pump.
- (6) Grounded electrode: This collects the charged nanofibers and completes the circuit.
- (7) Enclosure: A chamber where one can control temperature and humidity.

Such parameters can be controlled in a very stringent manner given voltage, spinning distance, collector distance, flow rate, and other environmental conditions for tuning the properties of the nanofibers produced for tissue engineering, filtration, and drug delivery ^[16,17].

2.2. Components of an electrospinning setup

The experimental setup for electrospinning are as follows (**Figure 1**):

- (1) High voltage power supply: Supplies the electric field needed to electrospin the polymer solution into fine fibers. Voltages used are normally between 10 kV and 30 kV.
- (2) Spinneret: A nozzle or a needle, using which the polymer solution is spun. The diameter of the spinneret affects the diameter of the resulting nanofibers.
- (3) Syringe pump: The combination helps regulate the infusion of the polymer solution by the spinneret. It equally leads to uniform and controlled production of fibers.
- (4) Collector: This may be a grounded surface, flat plate, or rotary drum on which the nanofibers are collected. The shape and movement of the collector will determine the alignment and morphology of the fibers.
- (5) Polymer solution reservoir: These will hold the polymer solution and feed to the syringe pump crucial to the process are the properties and concentration of the solution.

- (6) Grounded electrode: It is meant to complete the electric circuit and collect the deposited charged nanofibers.
- (7) Enclosure: A chamber that controls environmental conditions such as temperature and humidity, which can have a huge influence on the electrospinning process and the properties of the obtained fibers ^[18,19].

2.3. Controlling parameters in electrospinning

- (1) Voltage: Determines the strength of the electric field and affects the formation of the Taylor cone and the fiber diameter.
- (2) Flow rate: Influences the rate at which the polymer solution is extruded, affecting the thickness and uniformity of the fibers.
- (3) Spinneret-collector distance: Impacts the stretching and thinning of the fibers as they travel to the collector.
- (4) Solution properties: Including viscosity, concentration, and conductivity, which affect the fiber formation and morphology.
- (5) Environmental conditions: Temperature and humidity can alter the solvent evaporation rate and the behavior of the polymer solution during electrospinning ^[20,21].

2.4. Applications of electrospun nanofibers

- (1) Tissue engineering: Nanofibers are capable of mimicking the ECM, hence supporting cell attachment, proliferation, and differentiation. They find applications in scaffolds for tissue engineering of bone, skin, nerve, and cardiac tissues.
- (2) Filtration: Because nanofibers have high surface areas to volume and very small pore sizes, they can suitably be applied in both air and water filtrations.
- (3) Drug delivery: Nanofibers can be loaded with drugs and provide controlled release profiles, hence increasing the efficacy of the drug delivery system.
- (4) Wound healing: Electrospun nanofibers have the potential to produce dressings facilitating the healing process by protecting from infections, supporting gas exchange, and absorbing fluids ^[22–24].

2.5. Advantages of electrospinning

- (1) High surface area: Nanofibers have a large surface area, beneficial for applications requiring high interaction with the environment or cells.
- (2) Porosity: The interconnected porous structure facilitates cell infiltration and nutrient exchange in tissue engineering.
- (3) Versatility: A wide range of polymers can be electrospun, allowing for customization of fiber properties to suit different applications.

2.6. Challenges and innovations

- (1) Uniformity and control: It is challenging to achieve homogeneity in fiber diameter and uniform scaffold properties.
- (2) Mechanical strength: Electrospun fibers can be too weak for high mechanical strength applications and require reinforcement.
- (3) Scale-up: Quality assurance is a continuing challenge in large-scale production.

(4) Multi-component and functionalized fibers: Research studies are focused on the development of composite fibers and the introduction of bioactive molecules to impart functionality.

(5) 3D structures: Developing techniques for the creation of three-dimensional electrospun structures for more complex tissue engineering applications. Refining the process of electrospinning and researching new materials and techniques have been helping researchers move the potential applications of electrospun nanofibers into an increasingly broad spectrum of fields.

2.7. Working principle

- (1) Solution preparation: A polymer solution or melt is prepared. The choice of the polymer, solvent, and concentration used is important since they will affect the viscosity, surface tension, and conductivity of the solution.
- (2) Solution loading: The polymer solution is loaded into a syringe equipped with a metal needle (spinneret).
- (3) High voltage application: A high-voltage power supply is applied to the needle. The voltage between the spinneret and a conducting collector serves to provide an electric field.
- (4) Jet formation: The voltage is increased until it reaches a point where electrostatic forces are stronger than the surface tension of the polymer solution, whereby a Taylor cone is formed at the tip of the needle. If the electrostatic force overpowers the surface tension, a charged jet of polymer solution is squirted off from the tip.
- (5) Stretching and thinning: The jet is gradually subjected to stretching and thinning as it moves toward the collector. This results in the evaporation of the solvent and the solidification of the polymer fibers.
- (6) Deposition: The fibers are deposited on a grounded collector, usually a flat plate, a rotating drum, or other structures that can provide a proper geometry for subsequent fiber alignment and properties ^[25].

2.8. Experimental setup

- (1) Syringe and needle (Spinneret): The polymer solution is held in a syringe connected to a metal needle. The needle serves as the spinneret from which the polymer jet is ejected.
- (2) High-voltage power supply: This is used to generate the electric field required for electrospinning. Typical voltages range from 5 to 30 kV.
- (3) Pump system: A syringe pump controls the flow rate of the polymer solution. The flow rate needs to be optimized based on the polymer and solvent used.
- (4) Collector: The collector is grounded and placed at a certain distance from the needle. The design of the collector can vary:
 - (5) Flat plate: For randomly oriented fibers.
 - (6) Rotating drum: For aligned fibers.
 - (7) Rotating mandrel: For tubular structures.
- (8) Environmental control: Some setups include a controlled environment chamber to regulate temperature, humidity, and air flow, as these factors can influence fiber formation and properties.

2.9. Process parameters

Several parameters can be adjusted to control the characteristics of the electrospun fibers:

- (1) Solution properties: Concentration, viscosity, surface tension, and conductivity of the polymer solution.

- (2) Voltage: The magnitude of the applied voltage affects the jet formation and fiber diameter.
- (3) Flow rate: The rate at which the polymer solution is fed to the needle.
- (4) Distance between needle and collector: Affects the flight time and, consequently, the fiber formation.
- (5) Collector type and speed: Influences fiber alignment and density.

Electrospinning is an advanced process for the fabrication of nanofibers by applying a high tension to a polymer solution. This method produces fine fibers, and these fibers are collected over a grounded surface. The properties of the resultant fibers for a specifically desired application could be easily tailored with the manipulation of the experimental parameters at a fine scale. Electrospinning is the process of creating extremely fine fibers by using an electric field to convey a polymer solution or melt onto a small droplet at the tip of a slender fiber. The general configuration of the electrospinning experiment is done with a syringe pump to control polymer solution liquid delivery, a high voltage power source that creates an electric field, a spinneret to extrude a polymer solution as a jet, a collector to store the fibers, and a grounded plate that closes the electrical circuit. First, the electrospinning polymer solution is injected by a syringe pump and then, under the influence of high voltage supplied from a power source, electrical charges are applied. The electrostatic forces are created by the repulsion of similar charges in the polymer solution, overcoming surface tension, and creating a jet from the solution, elongated and thinned towards the collector. Simultaneously, solvent evaporates and fine fibers are laid on the collector. Some important parameters affecting the electrospinning process include the concentration of the polymer solution, flow rate of the solution, applied voltage, tip-to-collector distance, and environmental conditions. All of these variables are tuned for tailoring the diameter, morphology, and properties of the final electrospun fibers for a plethora of uses in tissue engineering, filtration, and drug delivery.

2.10. Taylor cone in electrospinning process

In the process of electrospinning, nanofibers form through the Taylor cone. A high voltage is applied to a polymer solution or melt in this process. As the charged material jet travels toward the collector, the repulsion of like charges stretches it, making it increasingly thin in radius. The Taylor cone is a pointed projecting shape that forms at the jet tip when electrostatic forces overcome the surface tension of the polymer solution. The cone-shaped formation is named after Sir Geoffrey Ingram Taylor, who studied the behavior of electrified droplets in the early 1960s. The Taylor cone's formation is rather important since it determines the stability and morphology of nanofibers resulting from it. A stable cone shape in the process produces uniform and well-defined nanofibers, whereas if it is unstable, beads-on-a-string morphology or irregular fibers will form. That is to say, generally, the Taylor cone in the process of electrospinning acts as a very crucial factor that impinges on the quality and properties of the nanofibers produced and becomes an important focus of research and optimization in electrospinning technology ^[26–29].

2.11. Bending instabilities in electrospinning

Bending instabilities are widespread in this technique for manufacturing nanofibers. The instabilities occur when a charged jet is extruded from the spinneret tip with a polymer solution, getting bent as a result of various mechanisms: these range from the field intensity, the solution viscosity, and the flow rate. The jet, in its trajectory toward the collector, can be affected by whipping, buckling, or meandering motions, making the morphologies produced irregular. Bending instabilities are very important regarding both controlling and understanding electrospinning in order to produce uniform and nanofibers with high quality. Various researchers have provided

suggestions to overcome the bending instabilities, such as optimal choice of processing parameters, reducing the dielectric constant, coaxial spinnerets, use of additives in the polymer solution, and introduction of secondary electric fields in guiding the jet. By minimization of such instabilities in bending, the mechanical properties, porosity, as well as functionality of electrospinning nanofibers (for a wide array of applications including tissue engineering, filtration, and drug delivery) are hoped to be improved. In general, overcoming the bending instabilities found in the electrospinning pack is needed both for the advancement of the technology in general and to properly unleash the potential capability of materials based on nanofibers for its multiple applications ^[30,31].

2.12. Controlling parameters in electrospinning

One of the versatile techniques used in producing nanofibers is electrospinning, where electric fields are applied to a polymer solution. In this process, different parameters must be controlled to produce fibers with desired properties. Some of the most important parameters are polymer concentration, solution viscosity, applied voltage, flow rate, the distance between the needle tip and collector, and finally the environmental conditions like temperature and humidity. The polymer concentration can be varied to impact fiber diameter and morphology. Usually, larger diameters are the result of higher polymer concentrations. This then changes the viscosity of the solution affects the alignment of the fibers and, indirectly, their mechanical properties. The applied voltage influences the extent of stretching and thinning of the polymer jet that changes the fiber diameter. The flow rate affects the productivity and morphology of the fibers, higher flow rates give finer fibers. The distance between the needle tip and the collector should be optimized for controlling the fiber alignment and collection efficiency. Environmental conditions impact the drying and solidification of fibers, hence finally affecting the characteristics of the fibers. Those variables can be changed systemically by researchers or engineers to further fine-tune the features of the electrospun fibers to exhibit some desired properties relevant to applications related to biomedicine, filtration, or tissue engineering ^[32–34].

2.13. Modified electrospinning setups

Electrospinning is one of the most versatile techniques developed for the production of nanofibers from different materials. There may be some variations or enhancements in the modified electrospinning experimental setup compared to the traditional one for achieving a specific objective or improvement in the process. Some common modifications/enhancements are

- (1) Needle configuration
 - (a) Multi-needle setup: This configuration uses multiple needles to increase the production rate of nanofibers;
 - (b) Coaxial electrospinning: A technique where there is a needle within a needle for generating core-shell fibers that enable the encapsulation of materials therein.
- (2) Collector design rotating drum collector
Using this collector, the fibers are oriented in one direction, which may become very useful in applications that require oriented nanofibers.
- (3) Solution delivery system syringe pumps
They are used to control with high accuracy the flow rate of the polymer solution. Pressurized systems can enhance the uniformity in fiber diameters by providing consistent solution flow.
- (4) Environmental control

Humidity and temperature control can influence the evaporation rate of solvents and the morphology of fibers.

(5) Enclosed chambers

This may reduce contamination and permit more stringent control of environmental factors.

(6) Electric field mods

(a) Variable voltage: Voltage adjustment controls fiber diameter and morphology;

(b) Auxiliary electrodes: These may be used to guide the path of fibers, improve alignment, or form specific patterns.

(7) Material-specific adaptations

(a) Dissimilar solvent systems: For different polymers or composite materials;

(b) Additives: Like surfactants or nanoparticles to modify the properties of fibers.

(8) Post-treatment systems thermal treatments: Heat treatments applied after spinning enhance fibers' mechanical properties.

(9) Crosslinking or functionalization: This can modify the surface chemistry of the fibres. Such changes can be modulated according to specific research goals aimed at improving the uniformity of the fibers, increasing production rates, and achieving certain structural or functional features in the nanofibers. In this case, if there are specific goals or materials in mind, the setting-up can be tailored to meet such needs.

Modified electrospinning configurations include the following changes to the conventional electrospinning setup, either increasing fiber production efficiency or resulting in original structures of the produced fibers. One such modification includes the addition of a co-axial or multi-axial spinneret setup. This enables encapsulation of the core materials in the fibers, which will produce core-shell fibers having controlled release properties. Another modification is the incorporation of an electrospinning setup inside a controlled environment chamber, such as a glovebox or inert gas atmosphere, which allows for electrospinning of sensitive materials prone to reaction with moisture or oxygen. The modifications of the electrospinning parameters, in terms of voltage, flow rate, and collector speed, will also have an impact on the morphology and properties of the fibers. These may include the production of finer fibers, increased production rates, and structures with alignment. In general, modified electrospinning set-ups provide versatility and control of the electrospinning process; thus, they are capable of producing a wide variety of fibers with tailored properties for many tissue engineering, filtration, and even drug delivery applications ^[35,37].

3. Far Field Electrospinning

Far-field electrospinning is one of the techniques in the production of nanofibers by using an electric field that is imposed on a polymer solution or melt. Unlike conventional electrospinning, where the electric field is applied close to the spinneret, far-field electrospinning applies the electric field from some distance. This allows the formation of longer and more aligned nanofibers. In far field electrospinning, typically, a solution of the polymer is pumped through a spinneret toward a grounded collector while an electric field is applied over a longer distance. This stretches the polymer fibers and aligns them in flight toward the collector, ultimately forming nanofibers. Far field electrospinning offers advantages in terms of scalability and continuous production of nanofibers over large areas. It has a wide range of applications in tissue engineering, filtration, textiles, and drug delivery. Therefore, far field electrospinning is expected to be one of the prospective technologies for the production of advanced

materials with special functionalities since it enables the production of well-aligned nanofibers with controlled properties. Far Field Electrospinning (FFES) is a variant of the traditional electrospinning process wherein the distance between the needle and the collector is considerably increased to values over 20 cm. Therefore, it impacts the resultant nanofiber morphology and its properties at this extended distance ^[20,36].

3.1. Key features and benefits of Far Field Electrospinning

- (1) Enhanced fiber uniformity: Increasing the distance allows more time for the solvent to evaporate completely, leading to more uniform fibers with fewer defects or beading.
- (2) Greater fiber alignment: With a longer travel path, the fibers have more time to align due to the stretching and whipping motion caused by the electrostatic forces.
- (3) Reduction in jet instabilities: The extended distance can help reduce jet instabilities, which are common in close-proximity electrospinning setups, leading to more consistent fiber formation.
- (4) Customization of fiber properties: By varying the distance between the needle and the collector, as well as other parameters like voltage and flow rate, researchers can tailor the diameter and surface morphology of the fibers.
- (5) Applications in large-scale production: The technique can be advantageous in scaling up the electrospinning process, as the increased distance allows for a broader area of fiber deposition.

3.2. Challenges and considerations in Far Field Electrospinning

- (1) Need for precise control: Precise control over the solution properties (viscosity, conductivity), environmental conditions (temperature, humidity), and electrospinning parameters (voltage, distance) is crucial to achieve desired fiber characteristics.
- (2) Potential for fiber breakage: The longer travel distance may increase the likelihood of fiber breakage if the mechanical properties of the polymer are not well-suited to the setup.
- (3) Collection efficiency: Ensuring efficient collection of fibers over a larger area requires careful design of the collector system, which might include moving collectors or specific designs to focus the fiber deposition.
- (4) Electrical considerations: A higher voltage may be required to maintain a stable jet over the increased distance, which can raise safety concerns and require careful handling.
- (5) Experimental setup for Far Field Electrospinning: High-Voltage Power Supply: Typically ranging from 10-30 kV, depending on the solution properties and distance.
Needle-to-Collector Distance: Often set beyond 20 cm; exact distance depends on the desired fiber properties.
- (6) Solution delivery system: Syringe pumps or pressurized systems to control the flow rate of the polymer solution.
- (7) Collector design: Can be flat, rotating, or patterned, depending on the application and desired fiber orientation.
- (8) Environmental controls: Enclosed chambers with temperature and humidity control can help stabilize the process and improve fiber consistency.

4. Near Field Electrospinning

Near Field Electrospinning (NFES) is a technique used in nanotechnology to produce nanofibers with diameters ranging from a few nanometers to several micrometers. Unlike traditional electrospinning, which operates in the far-field regime, NFES works in the near-field region, where the distance between the spinneret and the collector is much shorter. In NFES, a high voltage is applied to a polymer solution or melt at the tip of a spinneret to create an electric field. The electric field induces a charge on the surface of the polymer, causing the polymer to form a Taylor cone. As the polymer jet is ejected from the cone, it elongates and solidifies into nanofibers as it travels towards the collector. The proximity of the collector to the spinneret in NFES allows for better control over the deposition of the nanofibers, resulting in improved alignment and patterning. This technique is used in various applications such as tissue engineering, filtration membranes, sensors, and drug delivery systems due to the high surface area and porosity of the nanofibers produced. Near Field Electrospinning (NFES) is a variation of the traditional electrospinning technique where the distance between the needle and the collector is significantly reduced, typically to a range of a few millimeters to centimeters. This close proximity enables precise control over the deposition of nanofibers, making NFES suitable for applications that require fine patterning and alignment of fibers^[38].

4.1. Key features and advantages of Near Field Electrospinning

- (1) High-precision fiber deposition: The short needle-to-collector distance allows for precise control over the placement of fibers, enabling the creation of well-defined patterns and structures.
- (2) Enhanced fiber alignment: NFES facilitates the production of highly aligned fibers due to the limited whipping motion of the jet, which is constrained by the reduced distance.
- (3) Micro/Nano-scale patterning: The technique is particularly useful for applications that require micro- and nano-scale patterning, such as in the fabrication of sensors, microelectronic devices, and tissue engineering scaffolds.
- (4) Reduced electrical requirements: Lower voltage levels are typically required in NFES compared to conventional electrospinning, as the electric field strength needed to initiate fiber formation can be achieved with a smaller gap.
- (5) Material versatility: NFES can be used with a wide range of materials, including polymers, composites, and even biomolecules, enabling the fabrication of functionalized and multi-material fibers.

4.2. Challenges and considerations

- (1) Process control: Maintaining stable jet formation and consistent fiber deposition requires precise control of process parameters, such as voltage, flow rate, and needle-to-collector distance.
- (2) Limited production scale: NFES is generally more suitable for small-scale production and research applications, as the deposition area is limited by the proximity of the setup.
- (3) Complex setup: The need for precise alignment and control systems can complicate the setup and operation, requiring careful calibration and adjustment.
- (4) Potential for needle clogging: The proximity and small orifice size can increase the likelihood of needle clogging, particularly with high-viscosity solutions.

4.3. Experimental setup for Near Field Electrospinning

- (1) High-voltage power supply: Lower voltage range compared to conventional electrospinning, typically around 0.5–2 kV.
- (2) Needle-to-collector distance: Ranges from a few millimeters to centimeters, depending on the desired precision and fiber properties.
- (3) Micro-manipulators: To position and control the needle or collector with high precision, enabling detailed patterning.
- (4) Substrate/Collector: Can include various materials, such as conductive or non-conductive substrates, depending on the application.
- (5) Solution delivery system: Often involves syringe pumps for controlled flow of the polymer solution.

NFES is particularly advantageous for applications in microfabrication, biomedical engineering, and the creation of complex fibrous structures with high precision.

5. Electromechanical spinning

Electromechanical spinning is a process combining electrical and mechanical elements to perform controlled spinning. It is used for a variety of purposes, from textile production processes to fiber manufacturing and nanotechnology. In relation to textile production, electromechanical spinning is used to produce yarns and threads. Electrical elements in spinning machines help control the parameters of speed, tension, and twist to produce yarns that are consistent and of high quality. The electromechanical spinning method is applied in producing nanofibers with well-controlled properties in nanotechnology. The application of electrical fields during spinning will help the researchers orient and align nanofibers, hence developing enhanced mechanical, electrical, or optical properties. The overall result of this process is that it provides a versatile and efficient method of producing fibers and nanomaterials with tailored characteristics, hence securing value for technology in a number of industries. Electromechanical spinning, otherwise referred to as electro-mechanical spinning or electro-spinning with mechanical assistance, is a hybrid technique combining the working principles of conventional electrospinning with mechanical spinning methods. This technique aims to increase the control of fiber morphology, alignment, and production efficiency by involving mechanical forces in addition to the conventional electrostatic forces used in electrospinning^[39].

5.1. Key features and principles

- (1) Combination of forces: In electromechanical spinning, both electrostatic and mechanical forces are used to draw the fibers. The mechanical forces can come from rotating drums, rollers, or other mechanical means that assist in stretching and aligning the fibers.
- (2) Improved fiber control: The mechanical component provides additional control over the fiber formation process, allowing for more precise manipulation of fiber diameter, alignment, and orientation.
- (3) Enhanced alignment and orientation: Mechanical forces help in aligning fibers, which is beneficial for applications requiring highly oriented fiber structures, such as in textiles, filtration, or reinforced composites.
- (4) Scalability: The integration of mechanical systems can enhance the scalability of the fiber production process, making it more suitable for industrial applications.

5.2. Applications

- (1) Textiles and fabrics: The technique is used to produce nanofiber fabrics with enhanced mechanical properties, uniformity, and specific functional properties.
- (2) Biomedical engineering: It can be used to fabricate scaffolds for tissue engineering with aligned fibers that mimic the structure of natural tissues, enhancing cell growth and tissue regeneration.
- (3) Filtration systems: Electromechanical spinning can create filter materials with precise pore sizes and high mechanical strength, useful for air and water filtration.
- (4) Composites: The technique is applied in producing composite materials with reinforced nanofiber structures, which improve the mechanical and thermal properties of the composites.

5.3. Experimental setup

- (1) High-voltage power supply: Similar to conventional electrospinning, a high-voltage power supply generates the electrostatic field required to draw the fibers.
- (2) Mechanical components: This can include rotating drums, rollers, or other mechanical stretching systems that assist in the drawing and aligning of fibers.
- (3) Solution delivery system: Syringe pumps or pressurized systems are used to control the flow rate of the polymer solution.
- (4) Collector: The collector may include additional mechanical components to assist in fiber alignment and collection. These can be stationary or moving, depending on the desired fiber orientation.
- (5) Environmental Controls: Controlling temperature and humidity is important to maintain the consistency and quality of the fibers produced.

5.4. Challenges and considerations

- (1) Complexity of setup: The integration of mechanical systems adds complexity to the setup, requiring precise calibration and maintenance.
- (2) Process control: Ensuring consistent fiber quality requires careful control of both the electrostatic and mechanical parameters.
- (3) Material limitations: Not all materials are suitable for electromechanical spinning, and the choice of materials can affect the feasibility and quality of the fibers produced.

6. Application of electrospun scaffold

Electrospun nanofibers are extremely versatile materials with a wide range of applications across various fields. Here are some of the key applications:

6.1. Biomedical applications

- (1) Tissue engineering: Electrospun nanofibers provide a scaffold that mimics the extracellular matrix, promoting cell attachment, growth, and differentiation, making them suitable for tissue engineering applications such as skin, bone, and vascular grafts.
- (2) Drug delivery: Nanofibers can be used to create drug delivery systems that allow for controlled release of pharmaceuticals. This can improve the efficacy and reduce the side effects of various drugs.
- (3) Wound healing: Due to their high surface area and porosity, electrospun nanofibers can be used in wound

dressings that facilitate faster healing and provide a barrier against infections.

6.2. Environmental applications

- (1) Filtration: Nanofibers are highly effective in air and water filtration systems due to their large surface area and small pore size, which allow them to trap very fine particles and contaminants.
- (2) Oil spill cleanup: Electrospun nanofiber mats can be used to absorb oil from water surfaces, providing a method for cleaning up oil spills efficiently.

6.3. Energy applications

- (1) Battery separators: Electrospun nanofibers are used in lithium-ion batteries as separators that prevent short circuits while allowing ions to pass through, enhancing battery performance and safety.
- (2) Fuel cells: Nanofibers can be used in fuel cells to improve the efficiency of the electrochemical reactions that generate electricity.

6.4. Textile and apparel

- (1) Smart textiles: Incorporating electrospun nanofibers into fabrics can impart special properties such as water resistance, breathability, and antimicrobial activity, leading to the development of smart textiles for clothing and medical applications.
- (2) Protective clothing: Nanofiber layers can be added to protective clothing to enhance their barrier properties against biological and chemical hazards without compromising comfort.

6.5. Sensors

Electrospun nanofibers can be used to develop highly sensitive sensors for detecting chemicals, biological agents, and physical parameters such as pressure and temperature. Their high surface area-to-volume ratio makes them particularly effective in sensing applications.

6.6. Agriculture

In the controlled release of fertilizers and pesticides, nanofibers can be engineered to release fertilizers and pesticides in a controlled manner, improving efficiency and reducing environmental impact.

6.7. Cosmetics

In skin care products, electrospun nanofibers can be used in face masks and other skin care products to deliver active ingredients more effectively to the skin.

6.8. Food industry

In terms of food packaging, nanofibers can be used in food packaging to enhance the shelf life of products by providing better barriers to oxygen and moisture and incorporating antimicrobial agents.

6.9. Electronics

In terms of the flexible electronics, electrospun nanofibers are used in the development of flexible electronic devices, such as wearable sensors and flexible displays, due to their excellent mechanical properties and conductivity.

7. Conclusion

In summary, scaffolds in tissue engineering offer a temporary matrix guiding cell attachment, growth, and differentiation toward the overall growth of a new tissue. The scaffolds are fabricated from naturally occurring and synthetic polymers whose material characteristics such as biocompatibility, biodegradability, mechanical strength, and porosity can be attained. Development of more complex and functional scaffolds has been enabled by advancements in scaffold design and manufacturing methods for tissue engineering applications like electrospinning. It offers a versatile technique to produce highly porous, larger surface area scaffolds in the form of nanofibers, which can potentially mimic the extracellular matrix of different tissues. Voltage, flow rate, and distance from the spinneret to the collector can be manipulated to get fibers with the desired characteristics for applications in tissue engineering, filtering, wound healing, and drug release. Presently, consistency and mechanical strength have remained two big challenges in this field, and thereby in scale-up; innovations include multicomponent fibers, functionalized fibers, creating 3D structures. Electrospinning is gaining increasing interest in the field of regenerative medicine due to its versatility and suitability for the fabrication of nanotextured fibrous scaffolds. One of the main limitations of this technique is that of the enhanced bending instability, which is responsible for buckling/curling and leads to the formation of under-controlled tortuous fibrous morphologies, which may compromise the fidelity of the final nanofibers.

Generally, several attempts have been made to reduce these instabilities by modulating the process parameters within their optimal range or by using coaxial spinnerets. Understanding and control of these instabilities have huge potential to improve mechanical properties, porosity, and functionality of electrospun nanofibers for tissue engineering, filtration, and targeted drug delivery. Modified electrospinning setups allow for flexibility and control in the process in producing unique fiber structures and improved fiber production efficiency. Far-field electrospinning made the manufacture of long, straighter oriented nanofibers possible, which immediately found applications in tissue engineering, filtration, textiles, and drug delivery. Near-field electrospinning provides very high precision of fiber deposition and increased alignment for the dispensing of micro- and nano-scaled fibers for patterning applications. Electromechanical spinning, without deviating from the synergistic electrical and mechanical effects, produces fibers with controlled properties, so its applications are found in textiles, biomedical engineering, and composites. Electrospun nanofibers are found to have taken up an application since the last few decades in such vital areas as biomedical, environmental, and energy, in textile and apparel industries, and in sensors; agriculture; cosmetics; food packaging; and the like.

Disclosure statement

The authors declare no conflict of interest.

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Identification of Immune-Related Feature Genes in Ovarian Cancer Using Bioinformatics and Analysis of Immune Cell Infiltration

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Abstract: *Objective:* To identify immune-related feature genes in ovarian cancer through bioinformatics analysis and perform immune-related investigations, which hold significant value for the early diagnosis and prevention of ovarian cancer. *Methods:* Bioinformatics analysis was utilized to identify immune-related feature genes in ovarian cancer. The GSE18520 and GSE40595 datasets were downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) based on the gene expression comprehensive database, and the corresponding platform's chip probe information was retrieved. GSE18520 served as the training set, and GSE40595 served as the validation set. A total of 2660 immune response genes (IRGs) were obtained from the ImmPort database (<https://www.immport.org/home>). Immune genes were screened and analyzed for feature genes using the “limma” package of R (4.2.1) software, and the results were visualized in a heat map. LASSO regression analysis and ssGSEA analysis were conducted to investigate the distribution of immune cell infiltration. Changes in regression coefficients of different genes in the model were also analyzed. *Results:* Five key genes—*CLEC4M*, *DEFB1*, *LCN2*, *PTH2R*, and *LGALS2*—were identified, and the correlation between these key genes and immune cells was analyzed. *Conclusion:* The findings indicate that *CLEC4M*, *DEFB1*, *LCN2*, *PTH2R*, and *LGALS2* are significantly associated with various immune cell types, suggesting that these genes may regulate immune cell behavior and influence disease progression. This bioinformatics study provides a foundation for potential therapeutic targets in ovarian cancer; however, further clinical and experimental studies are required to validate the findings.

Keywords: Ovarian cancer; Immune cells; Bioinformatics; Genes

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1. Introduction

Ovarian cancer (OC) is one of the leading causes of death among gynecologic malignancies^[1]. Due to the lack of distinct early symptoms, OC is often diagnosed at advanced stages. Reliable diagnostic markers and early detection methods remain insufficient, emphasizing the need to improve early recognition of OC among health professionals

and the general female population^[2]. Therefore, identifying early warning indicators for OC prognosis and further exploring its molecular mechanisms can provide critical theoretical guidance for early intervention^[3].

Over the past decade, progress in chemotherapy has been slow in improving the prognosis of OC, prompting increased research into molecular-targeted therapies. Similar to other cancer types, OC exhibits significant heterogeneity across different subtypes and individual tumors, posing major challenges to the effectiveness of targeted drug therapies^[4]. This heterogeneity, a hallmark of many cancers including OC, has potential predictive value for survival outcomes following chemotherapy, particularly in high-grade serous ovarian cancer^[5].

In China, the prevalence of OC has shown a significant upward trend over the past 30 years, with a notable acceleration in the last five years. The most affected population consists of women over 40 years of age, particularly postmenopausal and elderly women^[6]. It is projected that the number of OC patients in China will continue to rise at a rate surpassing the global average over the next decade. Currently, surgical intervention remains the primary treatment for OC, serving to confirm tumor type and disease stage^[7]. Postoperative chemotherapy with platinum and taxane drugs is then administered. In the past two decades, the rapid development of immunotherapy has introduced new possibilities for OC treatment, with the screening of potential immunotherapeutic drugs and the provision of adjuvant immunotherapy offering the potential to extend patient survival^[8].

Bioinformatics, an interdisciplinary field combining biology, information science, and statistics, plays a critical role in cancer research^[9]. It encompasses the processing and quality control of raw sequencing data, variant detection and annotation, integration of diverse molecular data types, visualization, and the generation of interpretable data reports. In recent years, bioinformatics has been widely applied in cancer diagnosis and treatment, driven by the need to convert biological data into actionable knowledge^[10]. The integration and analysis of bioinformatics-generated big data are essential for personalized healthcare and genomics, establishing bioinformatics as a cornerstone of precision oncology^[11].

Given the advancements in bioinformatics, future research on immune-related genes in OC is of great importance. In this study, the ovarian cancer dataset (accession number GSE18520) was retrieved from the Gene Expression Omnibus (GEO) database. Immune-related genes were screened, and characteristic genes were analyzed using R software packages such as “limma,” with the aim of elucidating the pathogenesis of OC and providing new references for early diagnosis and treatment strategies.

2. Materials and methods

2.1. Data download and processing

Microarray or high-throughput data were downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) using “ovarian cancer” as the keyword, with the species set to “*Homo sapiens*.” The dataset was required to include both normal and disease samples. Datasets GSE18520 and GSE40595, along with their corresponding platform chip probe information, were retrieved and downloaded. GSE18520 was designated as the training group, while GSE40595 served as the validation group. During the conversion of probe IDs to gene symbols, if multiple probes corresponded to a single gene symbol, the average expression level was calculated to represent the gene expression level. This conversion was performed using Perl (version 5.10.1). Additionally, 2660 immune response genes (IRGs) were obtained from the ImmPort database (<https://www.immport.org/home>).

2.2. Differential expression analysis

Differential expression analysis was conducted using the “limma” package in R (version 4.2.1). Differentially expressed genes (DEGs) were identified based on an adjusted P -value < 0.05 and an absolute value of the \log_2 fold change ≥ 1 ($|\log_2FC| \geq 1$). Heatmap visualization of DEGs was performed using the “pheatmap” package.

2.3. Screening of immune-related feature genes in ovarian cancer

Two machine learning algorithms, Support Vector Machine Recursive Feature Elimination (SVM-RFE) and Least Absolute Shrinkage and Selection Operator (LASSO), were employed to predict immune-related feature genes in OC. The Lasso regression algorithm was constructed using the “glmnet” package, with ten-fold cross-validation to determine the optimal λ value. L1 regularization was applied to enhance prediction accuracy and facilitate feature gene selection. SVM-RFE, a supervised learning algorithm commonly used for classification and regression analysis, was used to score genes and iteratively select those with strong classification performance. The “e1071” package was utilized to implement the SVM-RFE algorithm.

2.4. Validation of feature genes and evaluation of diagnostic accuracy

The expression levels of feature genes were validated using the GSE40595 dataset obtained from the GEO database. The “limma” package was used to extract the expression levels of feature genes, and differential analysis was performed with thresholds of $|\log_2FC| \geq 1$ and adj. P -value < 0.05 . Violin plots of differentially expressed genes were generated using the “ggunchained” package. The diagnostic performance of each feature gene was evaluated by plotting receiver operating characteristic (ROC) curves using the “pROC” package.

2.5. Immune cell infiltration analysis

Single-sample gene set enrichment analysis (ssGSEA) was employed to compare gene expression data from samples with immune cell gene sets, calculating the relative abundance of immune cells in each sample. The ssGSEA algorithm was used to estimate the infiltration abundance of immune cells in normal and ovarian cancer samples, and immune cell differences between the two groups were visualized. Correlation analysis among immune genes, immune cells, and feature genes was conducted using the “ggcorrplot” package. A correlated network heatmap was generated using the “linkET” package.

2.6. Construction of the ceRNA network for feature genes

miRNAs associated with feature genes were predicted using the miRDB (<https://mirdb.org/>), TargetScan (http://www.targetscan.org/vert_72/), and miRTarBase (<http://mirtarbase.mbc.nctu.edu.tw/>) databases. The intersection of prediction results from these three miRNA databases was identified as the target genes of feature gene-related miRNAs. Long non-coding RNAs (lncRNAs) targeting key miRNAs were predicted using the spongeScan (<http://spongescan.rc.ufl.edu/>) database. A ceRNA network of mRNA-miRNA-lncRNA for feature genes was subsequently constructed.

2.7. Statistical analysis

All bioinformatics analyses were performed using R software (version 4.1.2, 64-bit). Independent sample t -tests were conducted for comparisons between the two groups.

3. Results

3.1. Differential expression analysis

A differential expression gene analysis was conducted to identify genes significantly differentially expressed between disease and normal groups. Using a P -value threshold of less than 0.05 and an absolute log fold change greater than 1 as screening criteria, 483 key genes were identified. These genes exhibited significant expression differences between the groups, indicating their potential roles in the disease's onset and progression. To visualize the expression patterns of these key genes, a heatmap was generated, illustrating their expression differences across samples (see **Figure 1**). The heatmap revealed distinct expression patterns between the disease and normal groups, establishing a foundation for subsequent in-depth analyses.

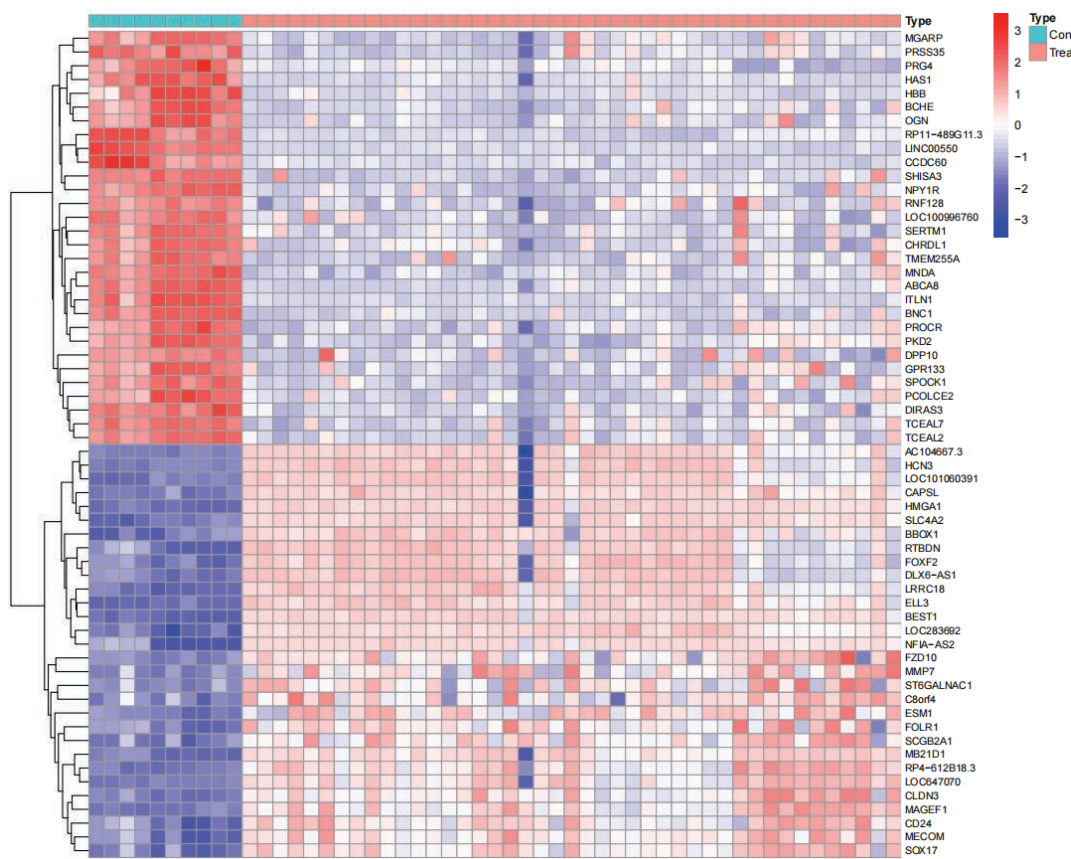


Figure 1. Heatmap of key genes. This heatmap displays the expression profiles of the 483 key genes with significant differences between the disease and normal groups. Each row represents a gene, and each column represents a sample. Red indicates gene upregulation, while blue indicates downregulation. The distinct expression patterns highlight the potential involvement of these genes in disease progression.

3.2. Key gene selection

To identify core disease-related genes among the DEGs, an intersection analysis was performed between the DEGs and known immune-related feature genes. This analysis identified 51 genes implicated in immune-related pathways (**Figure 2**). Further screening using the LASSO regression model narrowed the selection to five key genes: *CLEC4M*, *KCNH2*, *AKT3*, *TNFRSF8*, and *FCN1* (see **Figures 3** and **4**). These genes are hypothesized to play critical roles in immune regulation and disease progression, forming the focus of subsequent investigations.

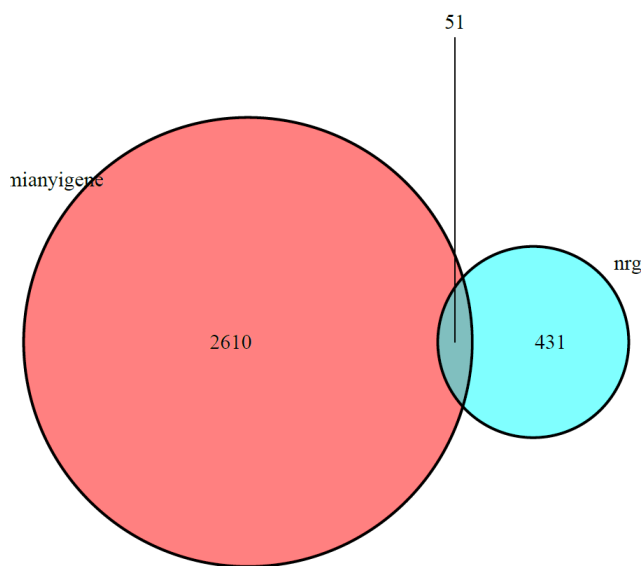


Figure 2. Intersection genes. The left side shows differentially expressed genes, while the right side displays known immune-related characteristic genes. An intersection analysis identified 51 key genes linked to immune-related pathways.

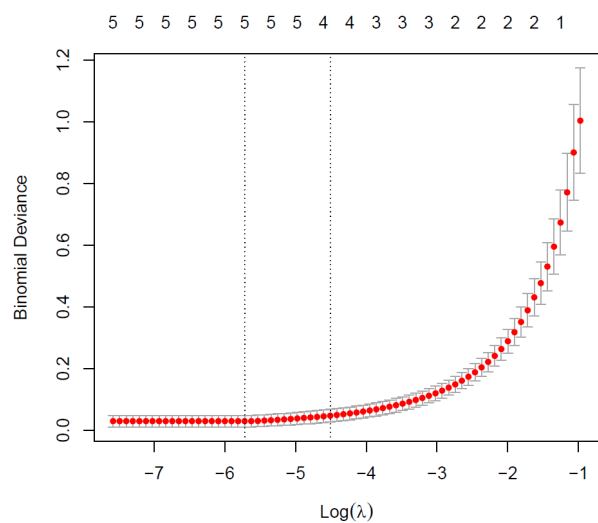


Figure 3. LASSO regression model selection parameter curve. This figure illustrates the relationship between the penalty coefficient (λ) and model performance in the LASSO regression analysis. The x-axis represents logarithmic λ values, and the y-axis shows the binomial deviance. Red dots indicate model deviations at different λ values, while the vertical dashed line identifies the optimal λ for gene selection.

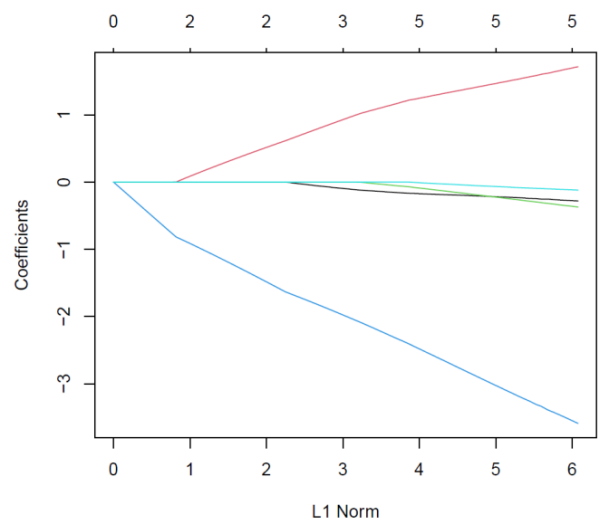


Figure 4. LASSO regression coefficient path diagram. This diagram depicts the changes in regression coefficients of genes as the penalty parameter (λ) varies. The x-axis represents the L1 norm (λ values), and the y-axis represents regression coefficients. Different colored lines correspond to different genes. The five identified key genes are those whose coefficients remain significant as λ increases.

3.3. Immune correlation analysis

To evaluate the relationship between the selected key genes and immune cells, ssGSEA was applied to assess immune cell infiltration. The analysis revealed significant differences in the infiltration levels of multiple immune cell types between the normal and disease groups. For instance, activated CD4 T cells and mast cells showed notable variations in infiltration levels (see **Figure 5**), suggesting their involvement in disease onset and progression.

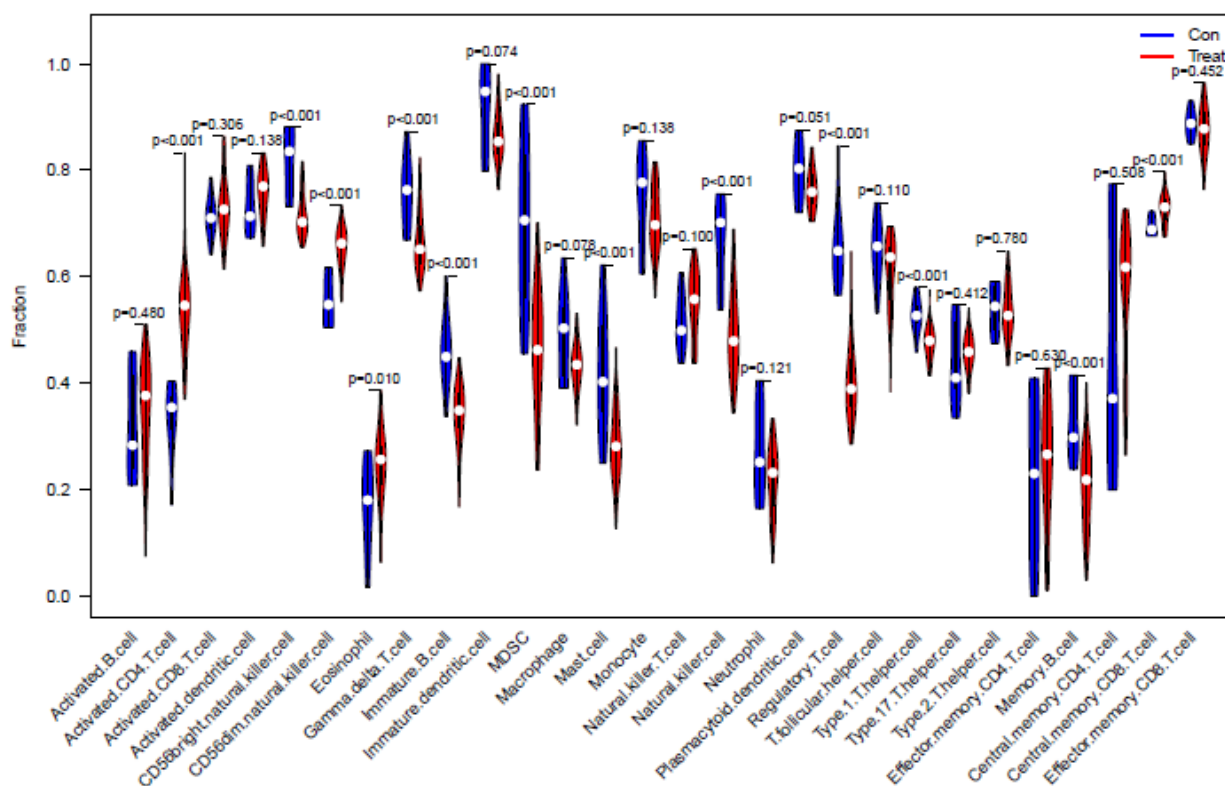


Figure 5. Immune cell infiltration distribution (ssGSEA analysis). This figure illustrates the infiltration levels of various immune cell types between the disease and normal groups. The x-axis represents immune cell types, and the y-axis denotes infiltration proportions. The *P*-values above each cell type indicate the significance of the differences.

Correlation analysis was also conducted to investigate the associations between the five key genes and various immune cell types (see **Figure 6**). The results highlighted a strong correlation between the *KCNH2* gene and several immune cell types, particularly immature dendritic cells and natural killer cells. These findings suggest that the selected key genes may influence disease progression through the modulation of immune cell behavior. This analysis provides valuable insights into the immune mechanisms underlying the disease and highlights potential diagnostic and therapeutic targets.

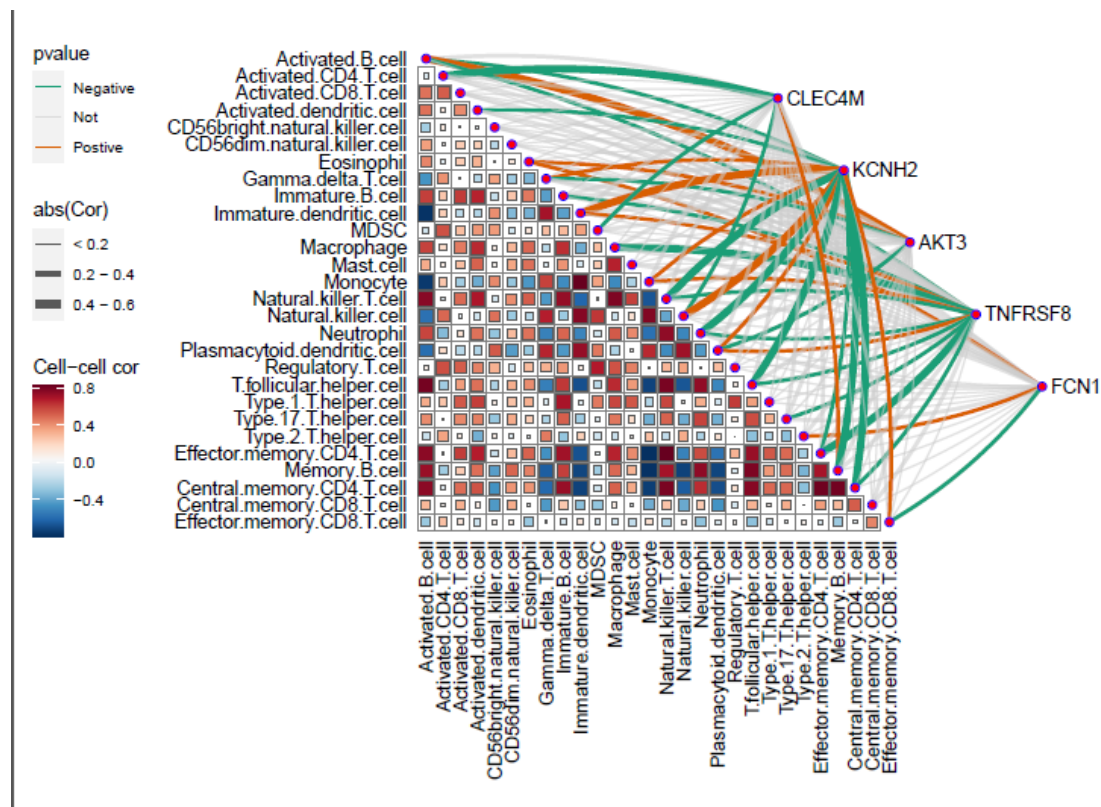


Figure 6. Correlation analysis between key genes and immune cell types. This figure shows the correlation between the five key genes and immune cell types. Line colors represent the correlation direction (orange for positive, green for negative), and line thickness indicates correlation strength. The KCNH2 gene exhibited significant correlations with multiple immune cell types, emphasizing its potential role in modulating immune cell behavior.

4. Discussion

Increasing evidence highlights the critical role of interactions between tumor cells and the tumor microenvironment, particularly the immune microenvironment, in tumor progression^[12]. This study utilized bioinformatics to analyze 2,660 immune-related genes from the GSE18520 dataset. Several genes, including *C-type lectin domain family 4, member M* (*CLEC4M*), *beta-defensin 1* (*DEFB1*), *lipocalin 2* (*LCN2*), *parathyroid hormone 2 receptor* (*PTH2R*), and *lectin, galactoside-binding, soluble, 2* (*LGALS2*), were identified as being associated with OC.

CLEC4M was found to potentially play a significant role in the activation and response of immune cells such as T cells, lymphocytes, myeloid leukocytes, and macrophages, reinforcing its involvement in immune activity and its influence on the tumor microenvironment. Studies have demonstrated a correlation between *CLEC4M* and tumor progression^[13,14]. Analysis using the KMplot™ database has shown that *CLEC4M* overexpression is linked to recurrence-free, progression-free, and disease-specific survival in patients. *CLEC4M* overexpression inhibits the proliferation of Huh7 and PLC/PRF/5 cells while enhancing apoptosis by suppressing the Janus kinase 1/signal transducer and activator of the transcription 3 pathway, which is implicated in various tumor types^[15].

The beta-defensin family is integral to host defense against viral infections, with *DEFB1* recognized as a critical antimicrobial peptide in epithelial cells^[16]. Although *DEFB1* is known as a tumor suppressor gene, its

role in OC has not been previously reported ^[17]. This study suggests that further investigation into *DEFB1* could provide valuable insights and novel approaches for OC treatment.

LCN2, a member of the adipokine protein family, regulates processes associated with cancer cachexia in diseases such as lung, pancreatic, breast, and oral squamous cell carcinomas ^[18]. It has garnered attention as a therapeutic target for cancers, including OC, where its transcriptional activity may influence cancer cell invasiveness and angiogenic capacity ^[19,20]. Serum *LCN2* levels have shown potential as biomarkers for epithelial ovarian cancer, warranting further research to determine its utility in early diagnosis and improved sensitivity and specificity for identifying subgroups of OC ^[21–23].

The parathyroid hormone 2 receptor (PTH2R), concentrated in the endocrine and limbic regions of the forebrain, is a B1 G protein-coupled receptor involved in calcium transport, nociception, and wound healing ^[24,25]. Analysis has linked PTH2R with the tumor suppressor gene *MUMILI*, with higher expression levels observed in normal ovarian tissue compared to OC tissue ^[26]. Reducing PTH2R expression has been shown to inhibit OC growth, invasion, and metastasis ^[27]. Furthermore, PTH2R expression correlates with increased tumor mutational burden (TMB), suggesting its potential as a future biomarker for OC.

LGALS2, a member of the galactoside-binding galectin family, is associated with immune evasion and disease pathogenesis in conditions such as inflammatory bowel disease, coronary artery disease, and cancer ^[28]. *LGALS2* increases tumor-associated macrophages, and its inhibition via antibodies has been shown to reverse immunosuppression and prevent tumor growth ^[29–31]. Elevated *LGALS2* expression is linked to favorable prognoses in diseases like rheumatoid arthritis and thyroid and colorectal cancers ^[30,33]. However, its role in OC remains unclear and requires further investigation to elucidate its therapeutic potential.

5. Conclusion

In conclusion, this study identified immune-specific targets for OC through systematic and effective screening methods. The findings provide a theoretical framework for further understanding OC pathogenesis and propose new targets for clinical treatment. However, these results are derived from online databases, necessitating additional validation through animal and clinical studies. Such efforts could enhance the clinical diagnosis and treatment of OC and support the development of novel therapeutic agents.

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Disclosure statement

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Observations On the Use of Personalized Composite Flaps in Postoperative Repair of Eyelid Tumour Excision

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Abstract: *Objective:* To analyze the application effect of personalized composite flap technology in the postoperative repair of eyelid tumour excision. *Methods:* To retrospectively analyze the clinical data of 76 patients who underwent postoperative repair of eyelid tumour excision in the outpatient clinic of the Affiliated Hospital of Hebei University during May 2022–April 2024, and group them according to the method of repair. 38 patients who underwent local flap transfer repair were included in the control group, and the other 38 patients who underwent personalized composite flap repair were included in the observation group. *Results:* Eyelid defect repair in the patients of the observation group was significantly higher than that of the control group (78.95%) in the total effective rate of defect repair (97.37%), ($P < 0.05$); before repair, the difference between the two groups of patients in terms of eyelid fissure length difference and eyelid fissure height difference was insignificant ($P > 0.05$); after repair, the difference in the length of the eyelid fissure and eyelid fissure height difference of the patients of the two groups was significantly reduced, and the observation group was lower than that of the control group ($P < 0.05$); before repair, there was no statistically significant difference between the aesthetic function scores of the two groups ($P > 0.05$); after repair, the aesthetic function scores of the patients in the observation group were significantly higher than those of the control group, and the difference was statistically significant ($P < 0.05$); after the operation, the total incidence of complications such as ptosis, eyelid margin incision marks and suture disintegration in the observation group was significantly lower than those in the control group (26.32%), ($P < 0.05$). *Conclusion:* The application of personalized composite flap technique for postoperative repair of eyelid tumour excision is effective, which not only helps to reduce the difference in lid height and length and improve the appearance of the patients, but also greatly reduces the risk of complications such as ptosis, lid margin incision marks and suture disintegration, and is recommended to be widely used in the clinic.

Keywords: Eyelid tumour excision; Personalized composite flap; Local flap transfer repair; Repair outcome

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1. Introduction

Eyelid tumours are one of the most common diseases in ophthalmology and plastic surgery, including benign tumours such as blepharoplastoma and sweat gland tumours, as well as malignant tumours such as basal cell carcinoma and squamous cell carcinoma ^[1]. Since the eyelid plays an important role in the protection of the eyeball and the secretion and distribution of tears, the treatment of eyelid tumours not only focuses on the thoroughness of the tumour resection but also on the postoperative repair of eyelid function and appearance. Traditional local flap transfer repair has some limitations in functional recovery and aesthetic effect, especially in the face of medium to large defects, and the difficulty of repair has increased significantly ^[2]. The personalized composite flap technique has shown unique advantages in recent years. This technique combines the biological properties of different types of flaps, and through precise design and individualization, it is possible to achieve a high degree of restoration of the postoperative appearance while meeting the anatomical and functional requirements. Especially after eyelid tumour excision, personalized composite flap repair can effectively cover the defective area, restore the physiological function of the eyelid, and improve the patient's facial aesthetics, thus significantly improving the patient's quality of life ^[3]. Compared with conventional flap repair, the personalized composite flap has a more precise and flexible adaptation, which not only can be designed according to the extent and depth of the defect but also can be used to select the appropriate type of flap and donor area according to the patient's specific conditions, thus reducing the incidence of postoperative complications ^[4]. This study aims to analyze the advantages and effects of this technique in eyelid repair and to provide more scientific evidence to optimize the postoperative treatment of eyelid tumours and improve the overall recovery of patients.

2. Information and methodology

2.1. General information

The clinical data of 76 patients who received postoperative repair treatment for eyelid tumour excision in the outpatient clinic of Hebei University Hospital in May 2023–April 2024 were retrospectively analyzed, and grouped according to the method of repair they received, and 38 patients who received local flap transfer repair were included in the control group, and the other 38 patients who received personalized composite flap repair were included in the observation group. In the control group, there were 20 males and 18 females, with an age range of 35–78 years old, and a mean age of 56.3 ± 10.4 years old. Tumour types: lid xanthoma 5 cases, sweat gland tumour 6 cases, basal cell carcinoma 17 cases, squamous cell carcinoma 10 cases; in the observation group, there were 19 males and 19 females, with an age range of 34–79 years old, and an average age of (57.1 ± 11.2) years old. Tumour types: 4 cases of blepharocarcinoma, 7 cases of sweat gland tumour, 18 cases of basal cell carcinoma and 9 cases of squamous cell carcinoma. Comparing the general data of the two groups of patients, the difference was not statistically significant ($P > 0.05$).

Inclusion criteria: (1) Patients diagnosed as basal cell carcinoma, squamous cell carcinoma or other benign tumours of the eyelid by pathological examination, all of which are unilateral eyelid tumours and need to undergo restorative treatment after tumour resection; (2) Age between 34 and 79 years old; (3) Physical health condition allows to receive eyelid tumour resection and repair surgery; (4) Patients voluntarily participated in the study, informed and agreed to postoperative follow-up and efficacy observation.

Exclusion criteria: (1) Patients with tumours spreading to both eyelids or involving other important tissues such as the eyeball; (2) Patients with serious systemic diseases (such as uncontrolled diabetes mellitus, cardiovascular disease, etc.) who cannot tolerate surgery; (3) The tumour invades the deep orbital layer or the

surrounding important tissues, and the treatment cannot be completed by conventional resection and repair; (4) Patients who are unable to cooperate with postoperative follow-up and treatment effect evaluation.

2.2. Methodology

2.2.1. Control group

The autologous labial mucosa and adjacent flap transfer repair technique was implemented. Preoperatively, the need for repair is assessed based on the extent and depth of the patient's defect after eyelid tumour excision, and the area of the lip mucosa to be taken and the adjacent skin flap transfer is designed. The lower lip mucosa is often chosen as the donor area, while the adjacent skin is planned as the flap donor area.

- (1) Tumour excision: Total resection is performed according to the extent of the tumour, ensuring that the margins are thoroughly removed, and rapid intraoperative pathological sectioning is performed to confirm that there is no tumour residue at the margins.
- (2) Lip mucosa harvesting: An appropriate amount of mucosal tissue was excised from the medial side of the lower lip without tension, paying attention to the protection of lip function and appearance, and the excised mucosa was used to repair the blepharoplasty and mucosal defects of the eyelid.
- (3) Neighbouring flap transfer: According to the design, a flap from the neighbouring area was cut to ensure that the size and shape of the flap were suitable to cover the eyelid surface defect. The flap is transferred to the defect site after preserving the blood supply, and at the same time, it is integrated with the labial mucosal tissue.
- (4) Fine suturing: The labial mucosa and the flap are fixed to the defect area, and attention is paid to the precision of the suture to ensure that the postoperative tissue tension is moderate and the natural curvature of the eyelid margin is restored.
- (5) Postoperative treatment: Cover the wound and fix it, apply antibiotics to prevent infection, and follow up regularly to observe the healing of the donor and repair areas.

2.2.2. Observation group

According to the specific scope and location of the eyelid defect and the involvement of surrounding tissues, a personalized repair plan was formulated, and the composite flap technique was used for repair. During the implementation of the repair of the inner layer of eyelid defects, the lid plate and lid conjunctival layer were incised, the lid plate was vertically separated to the position of the fornix, and the tipped lid conjunctival flap was transferred to the area of the defect and sutured to the stump of the lid plate to complete the reconstruction of the inner layer. In patients with 1/3–1/2 of the defect in the lateral canthus and posterior layer, an orbital periosteal flap at the base of the lateral orbital rim is designed according to the defect. The periosteal flap needs to be cut upward obliquely and sutured to the lid stump to fill the posterior layer defect. In severe patients with more than 1/2 of the posterior layer defects, free palpebral mucosal grafts are used to ensure structural integrity and functional recovery. For defects in the anterior layer of the eyelid, a tipped flap was used. Different repair strategies were used for different parts of the eye, such as the upper eyelid flap for lower lid defects, the frontal lozenge flap for medial canthus defects, and the lateral temporal pedicled transposition flap for lateral canthus defects. Postoperatively, a pressure bandage was applied to the operated eye for 1–2 days, and the dressing was changed once a day until the stitches were removed 7–10 days after surgery. After discharge from the hospital, the patient was instructed to have a regular review and follow-up for any discomfort.

2.3. Observation indicators

- (1) Effectiveness of eyelid defect repair: Based on the recovery of eyelid structure and function after surgery, the effectiveness of repair is classified as cured, improved and ineffective, according to the following criteria:
 - (a) Cure: The defective area is completely repaired, and the eyelid shape is in harmony with the surrounding tissues, with a good natural appearance and no obvious scar. The function of the eyelid is normalized, with smooth opening and closing of the eyes and good eyeball protection.
 - (b) Improvement: The defective area is basically repaired, and the appearance of the eyelid is slightly abnormal, but basically matches with the surrounding tissues. Eyelid function is partially restored, eye opening and closing activities are mildly limited, tear distribution is slightly affected, and there are minor postoperative complications, but they do not affect daily life.
 - (c) Ineffective: Failure to repair the defective area, obvious abnormal appearance of the eyelid, morphological imbalance or severe scarring. Eyelid function is not restored or significantly impaired, with complications such as difficulty in opening and closing the eyes, impaired tear distribution, or even exposure to keratitis. Necrosis of the flap or grafted tissue, or need for re-surgical repair due to infection, etc. Overall effective rate = cure rate + improvement rate.
- (2) Improvement of lid fissure condition: Changes in the difference in lid fissure height and length before and after repair were recorded.
- (3) Evaluation of aesthetic repair results: Combined with the functional and morphological changes of the eyelids, the aesthetic effect of the repair was evaluated, and the total score was 2 points. If the eyelids are bilaterally symmetrical, there is no obvious difference in the length and height of the lid, and there is no inward turning, 2 points will be recorded; if the morphology and function have improved, but the depth of the marginal cut mark is ≥ 1 mm, 1 point will be recorded; if there is no improvement in the morphology and function, 0 points will be recorded. The higher the score, the more ideal the restoration effect.
- (4) Incidence of complications: Record the occurrence of postoperative complications such as ptosis, eyelid margin scars and suture disintegration in patients. Total incidence rate = Number of relevant complications / Total number of cases $\times 100$ percent.

2.4. Statistical methods

SPSS 20.0 statistical software was applied to analyze and process the relevant data. Measurement data were expressed as mean \pm standard deviation (SD) and compared with *t*-test; count data were expressed as (*n*/%) and compared with χ^2 test. The difference was statistically significant with $P < 0.05$.

3. Results

3.1. Comparison of the effect of eyelid defect repair between the two groups of patients

Eyelid defect repair in patients of the observation group was significantly higher than that of the control group in terms of the total effective rate of defect repair, and the difference was statistically significant ($P < 0.05$), as shown in Table 1.

Table 1. Comparison of the results of eyelid defect repair between the two groups (n, %)

Groups	Curable	Take a turn for the better	Null	Overall effectiveness rate
Control group (<i>n</i> = 38)	12 (31.58)	18 (47.37)	8 (21.05)	30 (78.95)
Observation group (<i>n</i> = 38)	18 (47.37)	19 (50.00)	1 (2.63)	37 (97.37)
χ^2				4.5373
<i>P</i>				0.0332

3.2. Comparison of improvement in lid condition between the two groups of patients

Before repair, the difference between the two groups of patients in terms of lid length difference and lid height difference was not significant ($P > 0.05$); after repair, the difference between the two groups of patients in terms of lid length difference and lid height difference was significantly reduced, and the observation group was lower than the control group, with a statistically significant difference ($P < 0.05$) (Table 2).

Table 2. Comparison of the difference in lid length and lid height between the two groups of patients before and after repair (mean \pm SD, mm)

Groups	Difference in lid length				Difference in lid height			
	Pre-restoration	After repair	<i>t</i>	<i>p</i>	Pre-restoration	After repair	<i>t</i>	<i>p</i>
Control group (<i>n</i> = 38)	2.32 \pm 0.42	1.76 \pm 0.32	6.5378	0.0000	2.51 \pm 0.69	1.67 \pm 0.49	6.1186	0.0000
Observation group (<i>n</i> = 38)	2.34 \pm 0.45	1.46 \pm 0.28	10.2353	0.0000	2.53 \pm 0.57	1.44 \pm 0.42	9.4901	0.0000
<i>t</i>	0.2003	4.3492			0.1378	2.1969		
<i>P</i>	0.8418	0.0000			0.8908	0.0312		

3.3. Comparison of aesthetic function between the two groups of patients before and after restoration

Before repair, there was no statistically significant difference between the aesthetic function scores of the two groups ($P > 0.05$); after repair, the aesthetic function scores of the patients in the observation group were significantly higher than those of the control group, and the difference was statistically significant ($P < 0.05$) (Table 3).

Table 3. Comparison of aesthetic function scores between the two groups of patients before and after repair (mean \pm SD, points)

Groups	Pre-restoration	After repair	<i>t</i>	<i>p</i>
Control group (<i>n</i> = 38)	0.93 \pm 0.24	1.52 \pm 0.20	11.6418	0.0000
Observation group (<i>n</i> = 38)	0.91 \pm 0.26	1.85 \pm 0.12	18.3240	0.0000
<i>t</i>	0.3484	7.5603		
<i>P</i>	0.7285	0.0000		

3.4. Comparison of the occurrence of postoperative-related complications between the two groups

Postoperatively, the total incidence of complications such as ptosis, lid margin cut marks and suture disintegration

was significantly lower in the observation group than in the control group, and the difference was statistically significant ($P < 0.05$) (Table 4).

Table 4. Comparison of the occurrence of postoperative-related complications between the two groups (n, %)

Groups	Drooping upper eyelid	Eyelid margin incision	Stitching is falling apart	Total incidence
Control group (n = 38)	3 (7.89)	5 (13.16)	2 (5.26)	10 (26.32)
Observation group (n = 38)	0	1 (2.63)	0	1 (2.63)
χ^2				8.6098
P				0.0033

4. Discussion

The eyelid plays an irreplaceable role in protecting the eyeball and maintaining the normal visual function, therefore, repair after eyelid tumour excision is not only necessary for restoring the appearance but also concerns the reconstruction of eyelid function. Scientific and effective repair techniques can maximize the restoration of the anatomical structure and dynamic function of the eyelid, ensure smooth opening and closing movements, avoid corneal exposure and secondary infections, and at the same time improve the patient's appearance, quality of life, and psychological state, which is of great significance for the patient's overall recovery [5]. However, traditional local repair techniques, such as autologous labial mucosa combined with neighbouring flap transfer, although able to fill the eyelid defect to a certain extent and restore partial function, still have many shortcomings [6]. Firstly, the texture of the labial mucosa is significantly different from that of the eyelid tissue, which may lead to poor eyelid dynamics after surgery, affecting the opening and closing of the eyes. Secondly, the strong dependence of neighbouring flap transfer on the donor tissue may lead to increased tension, significant scarring, and impaired function of the donor area. In addition, this repair method makes it difficult to achieve precise reconstruction of anatomical levels in the face of medium- to large-scale defects, which may lead to unnatural postoperative appearance and asymmetric morphology; for this reason, it is necessary to actively explore a more precise and efficient repair method.

The personalized composite flap technique is a combination of multiple flaps and grafts, individually designed for the patient's specific defects, to achieve a multilevel reconstruction of the eyelid structure [7]. The core mechanism of the technique is the combination of lid conjunctival flaps, periosteal flaps, free palpebral mucosal, and tipped flaps through fine anatomical dissection and tissue transposition, which allows for the precise repair of the inner and outer eyelid structures while restoring the dynamic function of the eyelid [7].

The results of this study showed that the results of eyelid defect repair, improvement of lid fissure, aesthetic and functional scores, and the incidence of related complications were significantly better than those of the control group, and the reasons for this were mainly due to the significant advantages of the personalized composite flap repair technique. Firstly, the design of the composite flap can accurately match the size and scope of the patient's defect to achieve personalized repair. Second, the anatomical integrity and physiological function of the eyelid can be restored to the maximum extent through the combined application of multiple tissue materials [9]. Thirdly, the technique is particularly effective in cosmetic repair, improving eyelid symmetry and reducing scar formation. Fourth, due to the adequate blood supply and strong tissue integration, composite flap repair can significantly reduce the incidence of postoperative complications and improve the long-term stability and aesthetic effect of the

postoperative repair^[10].

5. Conclusion

In conclusion, the application of a personalized composite flap repair technique in the postoperative repair of eyelid tumour excision has a positive clinical value in terms of repair effect, aesthetic effect, and reduction of the risk of complications.

Disclosure statement

The authors declare no conflict of interest.

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***FLCN* - A Promising Novel Prognostic Biomarker for Lung Adenocarcinoma (LUAD) Patients**

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Abstract: *Rationale:* The tumor suppressor *FLCN* gene mutations are the primary cause of the rare autosomal recessive genetic disorder known as Birt-Hogg-Dubé (BHD) syndrome. Early diagnosis of BHD is difficult since *FLCN* mutation-caused tumors can form in the skin, lungs, kidney, and other organs and are benign. These tumors generate a range of phenotypes. *Methods:* The UALCAN database was utilized to ascertain *FLCN* expression and methylation in LUAD. Additionally, using KM plotter, GEPIA2.0, and cBioPortal, respectively, the survival, validity, and mutation analysis of *FLCN* was ascertained in LUAD. Using STRING and DAVID tools, the pathway and gene enrichment were identified in the presence of *FLCN*. The muTarget database was used to identify the mutant genes. *Results:* The goal of the current study is to examine *FLCN* expression in LUAD tissues. In these patients with LUAD, the study compared the expression of *FLCN* to other clinic-pathological characteristics. When comparing LUAD patients' clinical parameters to those of normal control samples, *FLCN* expression was higher. Additionally, it was discovered that a higher expression of *FLCN* in LUAD patients was linked to a shorter overall and disease-free survival. Results of gene ontology and pathway analysis demonstrated that genes linked with *FLCN* are significantly co-expressed with *FLCN* and are involved in a wide range of distinct molecular functions, biological processes, and pathways. *FLCN* expression was also correlated with promoter methylation levels, genetic alterations, other mutant genes. This crucial information demonstrated the important function that *FLCN* plays in the initiation and expansion of LUAD. *Conclusion:* This research emphasizes how crucial genetic analysis is to the diagnosis and the therapeutic treatment of LUAD.

Keywords: Lung adenocarcinoma; Diagnosis; Treatment; Biomarker

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1. Introduction

Lung cancer is the most commonly diagnosed malignant tumor and the main cause of cancer-related death. It is the second most common cause of new cancer cases in both genders in the United States and the second greatest cause of cancer deaths in females worldwide ^[1,2]. Lung cancer accounts for more than 27% of all cancer-related

deaths globally, with non-small cell lung cancer (NSCLC) accounting for 80% of cases ^[2,3]. Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), often referred to as non-small cell lung cancer (NSCLC), are the most prevalent subtypes of lung cancer ^[4,5]. Among NSCLC histological types, lung adenocarcinoma (LUAD) is the most prevalent one. The overall lung adenocarcinoma survival rate is still low despite considerable advancements in lung adenocarcinoma therapeutic approaches, such as surgical treatment, target therapy, and early cancer identification ^[6]. Currently, cytology screening and imaging examination are sensitive cancer screening methods, however they are ineffective for early identification of lung adenocarcinoma ^[7,8]. Recently, the clinical results of molecularly targeted therapies for LUAD patients are encouraging ^[9,10], while drug resistance still blocks curing LUAD patients ^[11,12]. Moreover, over 50% of patients are still not able to gain a limited benefit from targeted therapy ^[13,14]. Thus, reliable clinical outcome prediction and early lung cancer identification depend on the establishment of early diagnostic and prognostic biomarkers.

The *FLCN* gene is a tumor suppressor gene also known as the folliculin gene. *FLCN* produces a protein that may aid in controlling cell development and other critical cell processes. Mutated variants of the *FLCN* gene may promote the formation of aberrant cells. Birt-Hogg-Dube syndrome (BHDS) is an inherited disorder associated with a defective *FLCN* gene. Patients with BHDS are more likely to develop kidney cancer, as well as skin and lung cancers. The human *FLCN* gene on chromosome 17p11.2 comprises 14 exons and encodes a highly conserved protein termed folliculin, which is a classic tumor suppressor that regulates cell growth and proliferation ^[15,16]. An increasing amount of data indicates that the pathogenesis of Birt-Hogg-Dubé (BHD) syndrome (OMIM: 135150), an autosomal recessive condition marked by benign tumors in the skin, lungs, kidneys, and other organs, is mostly caused by mutations in the *FLCN* gene ^[15]. Age affects the location, development, and course of benign tumors, which can also vary by race or ethnicity ^[17]. This heterogeneity makes early diagnosis of BHD syndrome difficult and raises the chance of benign tumors becoming malignant ^[18]. BHD syndrome is linked to the development of cutaneous hamartomas (fibrofolliculomas, FF), numerous lung cysts (LCs), spontaneous pneumothoraces, and renal cell carcinoma (RCC) ^[19]. The renal malignancies linked with BHD are primarily of the hybrid oncocytic/chromophobe subtype, followed by clear cell renal carcinomas ^[20]. Approximately 85% of patients with BHD syndrome have multiple FF, which are benign dermatological papules that primarily affect the face and upper torso. These papules typically do not show until beyond the age of 20 ^[21–23]. Furthermore, in individuals with BHD syndrome, the dermatologic findings could be the only presenting symptoms, thus it's critical to recognize them so that a more thorough systemic evaluation can be carried out.

This study evaluated *FLCN* mutations, expression levels, survival prognosis results, and utilitarian perspectives within the LUAD framework using bioinformatics. This study also looked into the relationship between *FLCN* expression and promoter methylation levels. Numerous databases were used in this investigation, including the UALCAN portal, the Kaplan-Meier tool, the STRING database for protein-protein interactions (PPI), the cBioPortal, the Gene Expression Profiling and Interactive Analysis (GEPIA2.0), the Database for Annotation, Visualization, and Integrated Discovery (DAVID), and the Cancer Genome Atlas (TCGA) informational index. A vast array of functional annotation tools is offered by DAVID to help interpret the biological significance of the lengthy gene list. This study's main contribution was figuring out the *FLCN* expression pattern in LUAD and its potential significance for the onset and management of cancer.

2. Materials and methods

2.1. Expression and methylation analysis by UALCAN

The UALCAN database offers quick access to the cancer multi-omics data gathered from more than 30 distinct cancer types thanks to its user-friendly interface and intuitive features ^[24]. It performs comprehensive analyses of gene expression, protein abundance, and patient survival across a range of malignant tumor types using a significant amount of data from The Cancer Genome Atlas (TCGA). Researchers may examine and illustrate gene expression patterns linked to various cancer stages, molecular subtypes, and patient socio-demographics using the intuitive UALCAN interface. In the current study, this useful measure was employed to evaluate *FLCN* expression at different phases of a given cancer growth, where this gene exhibits significant dysregulation and is strongly associated with a poor overall survival rate. Using the UALCAN web tool, the *FLCN* promoter methylation level in LUAD was ascertained. In addition, the study assessed *FLCN* promoter methylation data in diverse clinical contexts, accounting for the age, gender, race, and cancer stage of the patient.

2.2. Survival analysis by KM plotter

KM plotter is a widely used tool for overall survival (OS) and disease-free survival (DFS) ^[25]. This web-based platform examines the impact of particular genes on a patient's propensity to survive various tumor growth types by analyzing vast volumes of clinical data. Researchers can quickly identify prognostic biomarkers and assess the prognostic significance of gene expressions. The main interface of KM Plotter shows Kaplan-Meier survival curves, which offer information on the relationship between patient outcomes and gene expression. Researchers studying the relationship between patient survival and gene expression levels in gastric, ovarian, lung, and breast cancers can benefit from the use of the KM plotter. This study used the KM plotter tool to examine the impact of *FLCN* dysregulation on overall survival (OS) in LUAD patients.

2.3. Expression and survival validation by GEPIA2.0

A well-known web application called GEPIA2.0 is used to predict expression and assess the durability of genetic data ^[26]. GEPIA2 has broadened the assessment of gene expression from the gene to the transcript levels. It has 198,619 isoforms and 84 cancer subtypes. It also facilitates the analysis of a particular cancer subtype and their interrelationships. Moreover, different cancer subtypes may have different prognoses. Moreover, different cancer subtypes may have different prognoses. Furthermore, as single-cell sequencing has become more widely available, new evaluation standards have emerged. Using the GEPIA 2.0 data set, the relationships between *FLCN* expression and prognosis (OS and RFS) in patients with LUAD cancer were investigated. The relationship between *FLCN* expression and the prognosis (OS and DFS) of LUAD cancer was examined in this study using GEPIA2.0.

2.4. Mutational analysis by cBioPortal

Because of its user-friendly features, the cBioPortal database is mostly used for multidimensional cancer omics data processing ^[27]. It provides a user-friendly interface for exploring tumor mutations, gene expression, and other genomic data across different cancer types. The portal aims to bridge the gap between complex genomic data and cancer researchers by providing intuitive access to molecular profiles and clinical attributes. Users can interact with the cBioPortal through a simple and flexible interface, intuitive visualization options, and a programmatic web interface. In this study, this data was used to perform mutational analysis of *FLCN* across LUAD cancer.

2.5. STRING AND DAVID analysis

Using the Search Tool for Retrieval of Interacting Genes/Proteins (STRING) ^[28], a PPI network comprising genes enriched in *FLCN* was extracted in this study. It makes use of an abundance of data to help scientists make sense of the confusing web of interactions between proteins. The study added STRING to the process of making *FLCN* proteins. When compared to other datasets, the STRING data collection is highly regarded for its enhanced quality control, coverage, and high availability of PPI information. Gene expression patterns and literature are just two of the data sources that STRING integrates to provide a single, comprehensive quality score for every communication. It makes use of PPI from computational and experimental methods. The GO and KEGG keywords of *FLCN* and its enriched genes were examined using DAVID ^[29] with $P < 0.05$ denoting significant findings.

2.6. muTarget

muTarget, an open-access tool, is used for associating mutational status with gene expression alterations across different tumors ^[30]. Using this tool, different mutant genes responsible for expression alteration in the *FLCN* across LUAD were identified in this study.

3. Results

3.1. Expression analysis of FLCN in LUAD

Employing the UALCAN data set, we inspected the *FLCN* expression in LUAD and normal control samples (**Figure 1**). After further investigation, this study discovered that LUAD malignant growth cells had a low levels of *FLCN* expression than the normal control samples. The strong down-regulation demonstrated the direct correlation between *FLCN* expression and the proliferation of malignant LUAD cells. This discovery raises the possibility that *FLCN*, a therapeutic target or diagnostic marker for LUAD, may play a critical role in preventing the multiplication of malignancy.

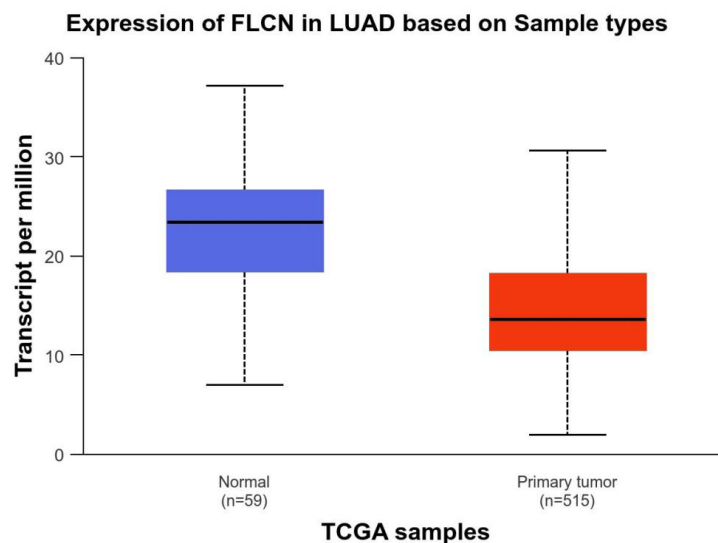


Figure 1. Expression profiling of *FLCN* in LUAD and normal tissue samples.

3.2. Expression analysis of FLCN in LUAD based on different clinical parameters

As a result, this study assessed *FLCN* in LUAD samples using a variety of clinical variables, including the

patient's age, gender, and race in addition to the particular cancer stage (**Figure 2**). When *FLCN* expression was first analyzed in different stages of cancer formation, the study discovered that, in comparison to normal control samples, LUAD samples demonstrated considerably low levels of *FLCN* expression in all stages (**Figure 2A**). Additionally, the study explored *FLCN* expression in LUAD patients and reported that all three racial groups, Asian, African-American, and Caucasian had significantly low levels of *FLCN* expression compared to normal control samples (**Figure 2B**). Furthermore, the study also examined the gender differences in *FLCN* expression in LUAD cancer patients and observed that both male and female patients had significantly low levels of *FLCN* than in normal control samples (**Figure 2C**). Lastly, the study investigated the association between patient age in LUAD and *FLCN* expression and revealed that among LUAD patients, *FLCN* was down-regulated in several age groups (**Figure 2D**). These results highlight *FLCN*'s potential as a helpful biomarker for diagnosis, prognosis and validate its significance in LUAD.

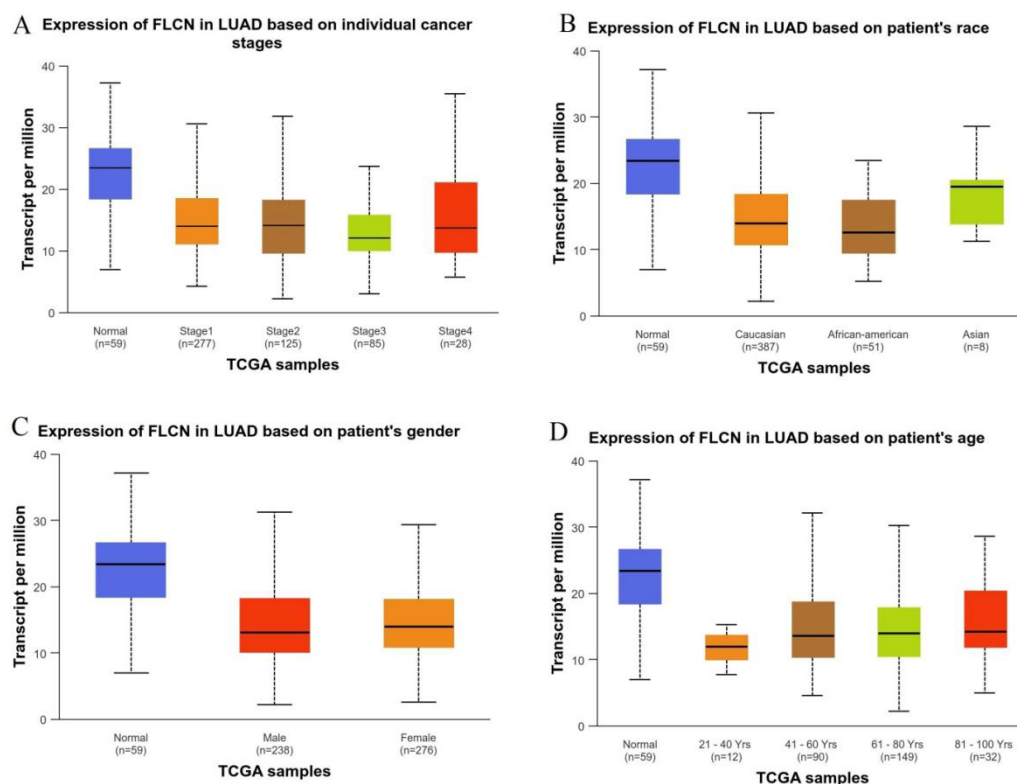


Figure 2. Expression of *FLCN* across different clinical parameters.

3.3. Validation of *FLCN* expression

The study used GEPIA2 to look at *FLCN* expression in LUAD cells and normal control samples. Compared to normal control samples, LUAD showed noticeably lower *FLCN* expression (**Figure 3A**). Additionally, GEPIA2 data set was used to analyze the relationship between *FLCN* expression and various stages of LUAD development. These results demonstrated a strong correlation between LUAD patient stages and *FLCN* expression. Furthermore, it was shown that the *FLCN* gene had the highest expression in stage IV and the lowest expression in stage III of the LUAD (**Figure 3B**).

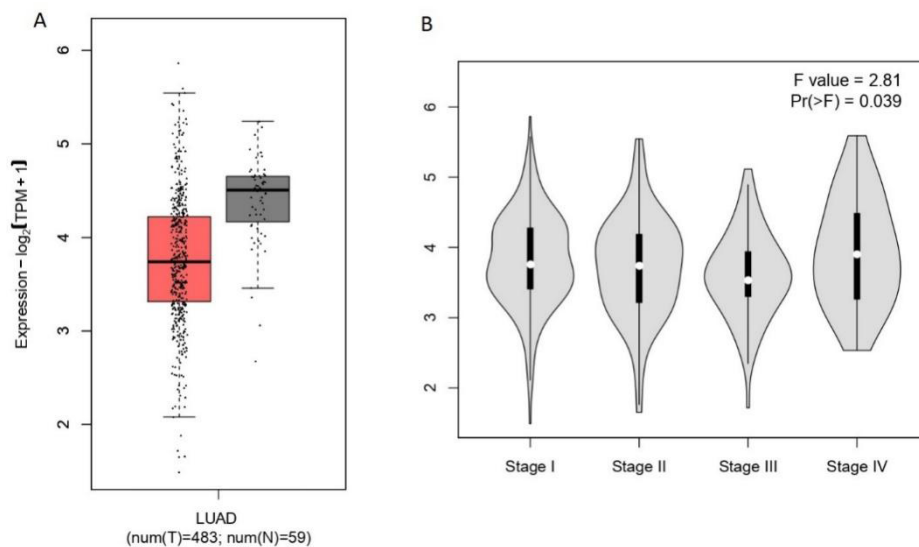


Figure 3. Validation of *FLCN* expression across different stages of LUAD.

3.4. Promoter methylation of *FLCN*

The study examined the *FLCN* promoter methylation levels in LUAD and normal control samples using the UALCAN online database. The results demonstrated that, in comparison to normal control samples, *FLCN* was hyper-methylated in LUAD samples (**Figure 4**). This result suggests that promoter methylation and *FLCN* expression are positively correlated in LUAD. This correlation demonstrated *FLCN*'s therapeutic potential in the pathophysiology of LUAD, suggesting that this cancer type may target *FLCN* for therapeutic therapies.

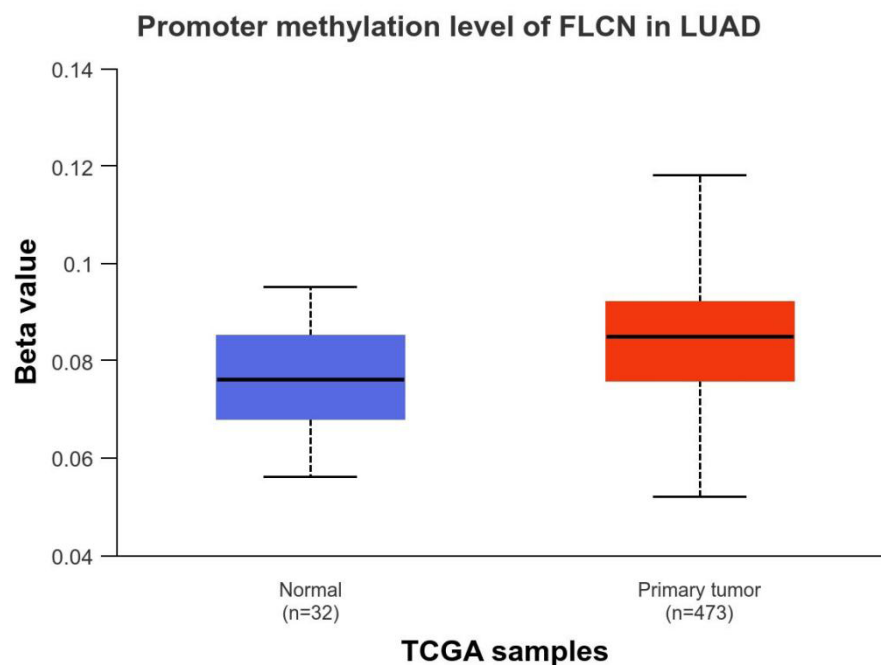


Figure 4. Promoter methylation pattern of *FLCN* in LUAD and normal control samples.

3.5. Promoter methylation of FLCN in LUAD across different clinical parameters

To learn more about the *FLCN* promoter methylation in LUAD, the study looked at several clinical parameters (**Figure 5**). In short, the study looked at *FLCN* promoter methylation and compared LUAD cancer progression phases to normal control data. Crucially, there were variations seen between phases; all four stages displayed hyper-methylation, in contrast to normal control samples (**Figure 5A**). Using the race of the LUAD patients as a criterion, the study examined *FLCN* promoter methylation. This study discovered evidence that all three racial groups (Asian, African-American, and Caucasian) had hyper-methylation in the *FLCN* promoter area as compared to normal control samples (**Figure 5B**). Subsequently, the *FLCN* promoter methylation was assessed, and the findings indicated hyper-methylation in both the male and female subjects (**Figure 5C**). Finally, the study examined the relationship between patient age and *FLCN* promoter methylation, finding that all age groups had methylation levels that varied across age groups and were significantly hyper-methylated when compared to normal samples (**Figure 5D**). These comprehensive analyses reveal the unexpected correlation between varying clinical parameters in LUAD and the *FLCN* promoter methylation, leading to a consistent pattern of hyper-methylation in the *FLCN* promoter methylation level in LUAD and highlighting its possible involvement in the genesis of LUAD cancer.

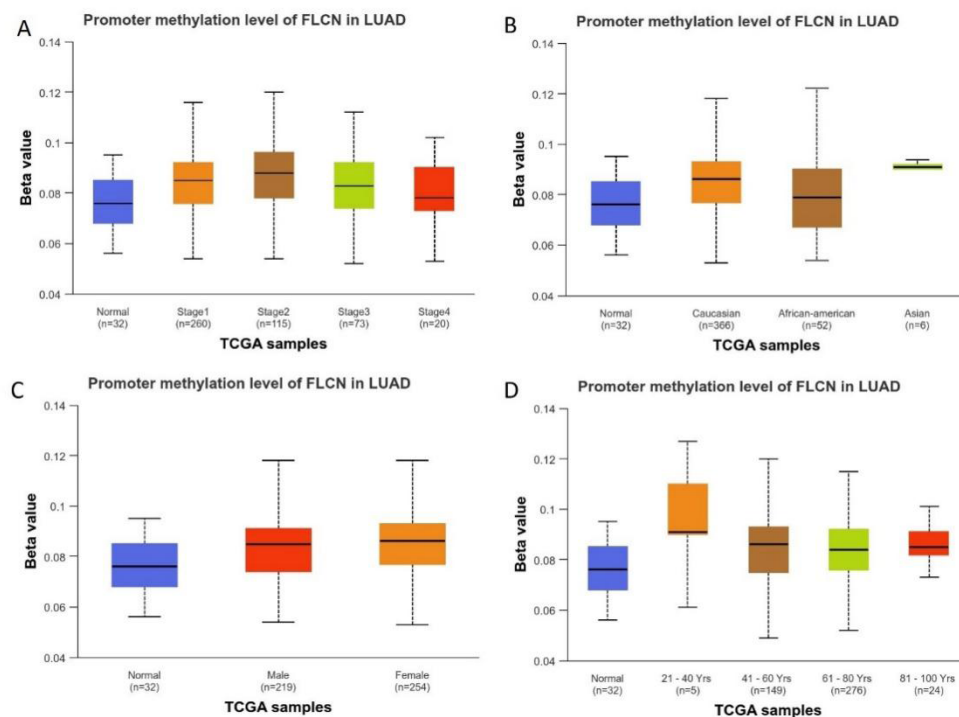


Figure 5. *FLCN* promoter methylation pattern across different clinical parameters.

3.6. FLCN survival analysis

The study used the KM plotter tool to create an assessment for overall survival (OS) and disease-free survival (DFS) in LUAD. These findings indicate that patients with LUAD who expressed low levels of *FLCN* had a better overall survival (OS) than those who expressed high levels of *CDON* (**Figure 6A**). Furthermore, in a disease-free survival (DFS) trial, LUAD patients with high *FLCN* expression performed worse than those with low *FLCN* expression. The studies emphasize the critical role that *FLCN* plays in determining patient survival

outcomes, highlighting its potential therapeutic utility as a prognostic marker in lung cancer therapy and implying its involvement in the course and progression of LUAD cancer.

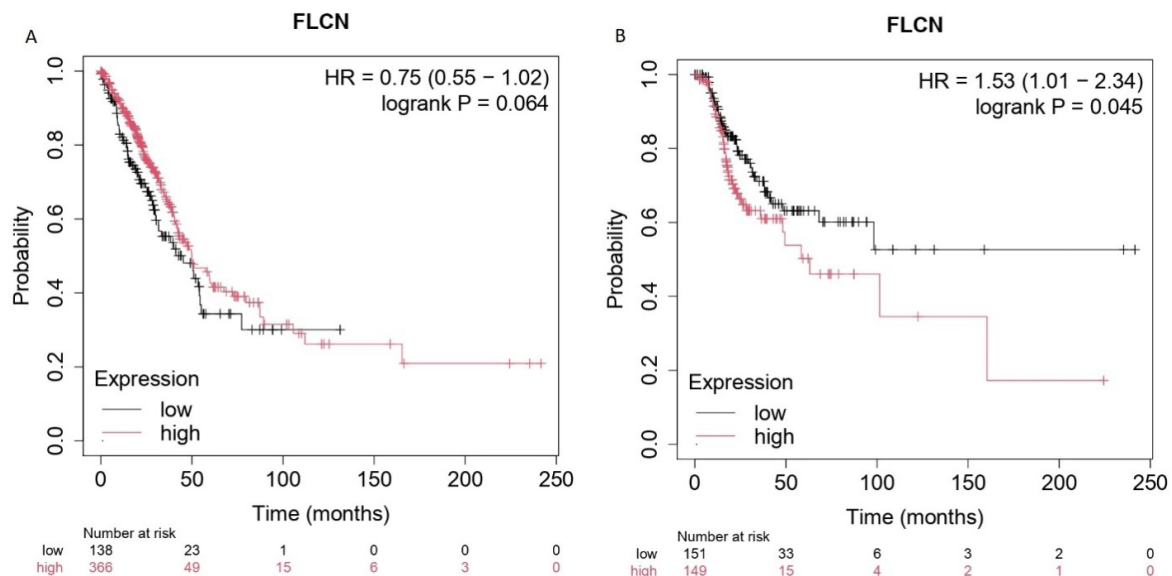


Figure 6. KM survival curve (OS, RFS) of *FLCN* in LUAD patients.

3.7. Survival validation of *FLCN*

Using the GEPIA2.0 informational tool, this study investigated the prognostic efficacy of *FLCN* expression in LUAD tumor progression. First, the LUAD patients were divided into two groups according to the levels of *FLCN* expression: low and high. In contrast to the high *FLCN* expression group, a low *FLCN* expression in LUAD was associated with great overall survival (OS) (**Figure 7A**). Next, in LUAD, a low *FLCN* expression level was linked with good disease-free survival (DFS) (**Figure 7B**). These results demonstrate the critical role that the *FLCN* gene plays in the initiation and expansion of LUAD cancer.

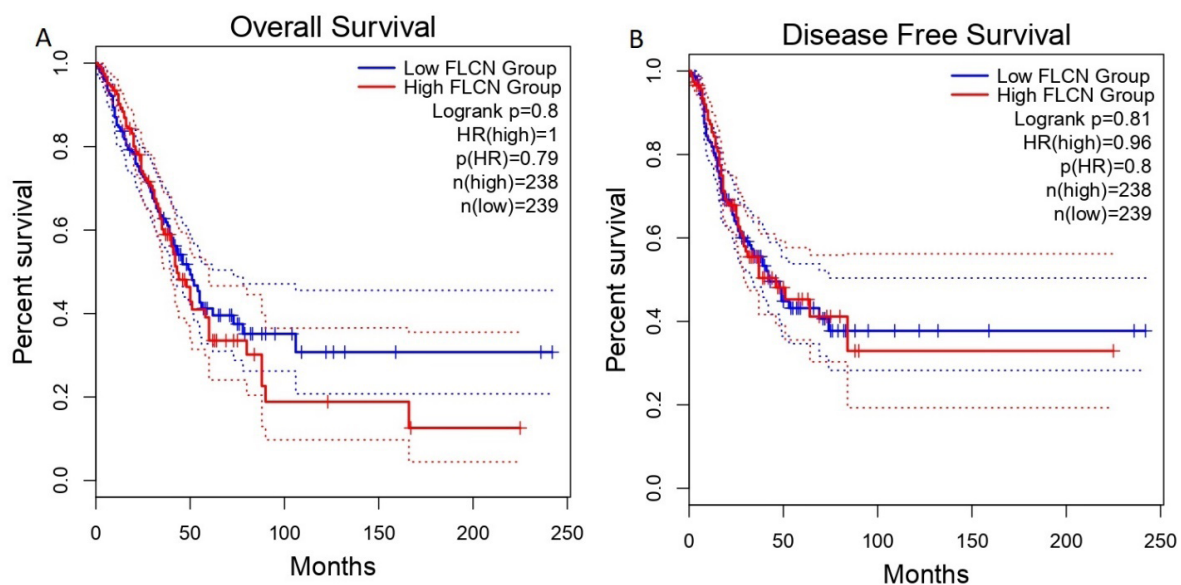


Figure 7. Survival curve (OS, RFS) of *FLCN* in LUAD patients.

3.8. Mutational analysis of FLCN

FLCN genetic changes in LUAD patients were observed using cBioPortal. The result show that genetic alterations in *FLCN* were only seen in 1% of LUAD samples. Among the chromosomal changes analyzed in LUAD were, truncating, in-frame and missense mutations (**Figure 8**), which may be essential to *FLCN* dysregulation in LUAD, even though genetic changes in *FLCN* are uncommon in LUAD.



Figure 8. Oncoplot of *FLCN* in LUAD cancer.

3.9. Protein-protein interaction (PPI) network of FLCN

The structural and functional relationships between the *FLCN* and DEG proteins were examined using the STRING program. The creation of PPI networks demonstrated the diversity of *FLCN* genes by revealing connections between the *FLCN* hub gene and 10 other genes, such as LAMTOR1, LAMTOR4, SLC38A9, RRAGC, RRAGA, LAMTOR2, RRAGB, RRAGD, FNP2, and FNP1. This suggests that *FLCN* is involved in many biological processes, performs a variety of tasks, and interacts powerfully with related genes.

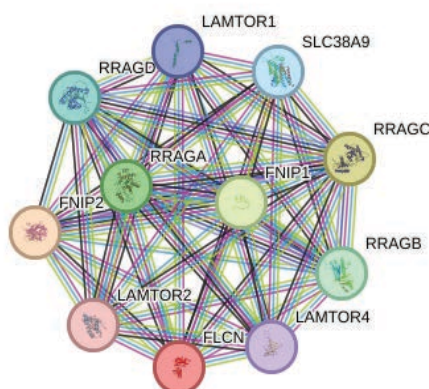


Figure 9. Protein-protein interactions of *FLCN*.

3.10. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The DAVID online tool was used to functionally annotate DEGs. The KEGG pathway-enriched genes and their possible GO (Gene Ontology) classification were studied using terms indicating biological processes, molecular activities, and cellular components related to KEGG pathways. The gene ontology BP analysis revealed that the GEGs are enriched in negative regulation of TORC1 signaling (GO:1904263), positive regulation of TOR signaling (GO:0032008), cellular response to amino acid stimulus (GO:0071230), cellular response to starvation (GO:0009267), protein localization (GO:0008104) (**Table 1** and **Figure 10A**). The gene ontology CC analysis revealed that the GEGs are enriched in FNIP-folliculin RagC/D GAP (GO:1990877), lysosomal membrane (GO:0005765), lysosome (GO:0005764), Gtr1-Gtr2 GTPase complex (GO:1990131), regulator complex (GO:0071986) (**Table 2** and **Figure 10B**). The gene ontology MF analysis revealed that the GEGs are enriched in GTPase binding (GO:0051020), molecular adaptor activity (GO:0060090), protein-membrane adaptor activity

(GO:0043495), guanyl-nucleotide exchange factor activity (GO:0005085), GTPase activity (GO:0003924) (**Table 3** and **Figure 10C**). The analysis of KEGG enrichment pathways showed that DEGs are involved in mTOR signaling pathway (hsa04150), autophagy – animal (hsa04140), shigellosis (hsa05131) (**Table 4** and **Figure 10D**).

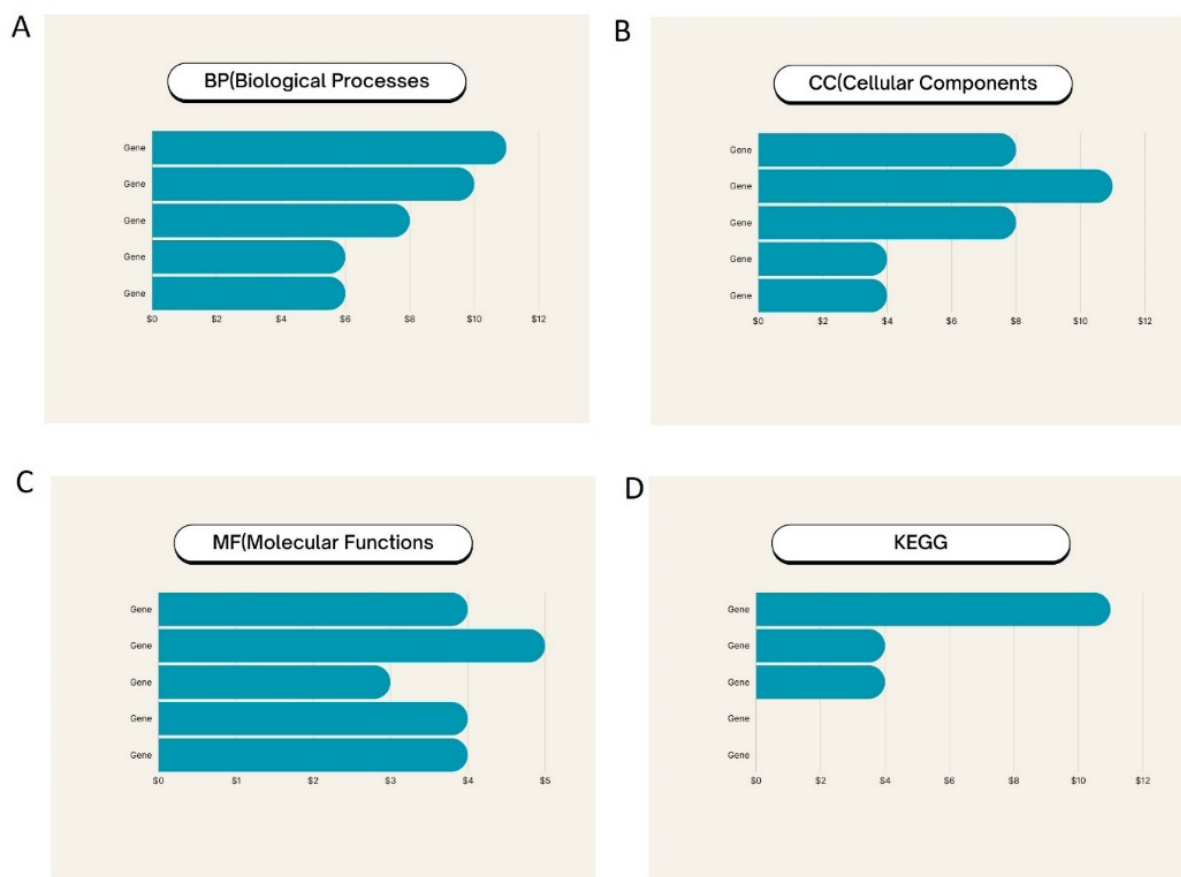


Figure 10. GO and KEGG analysis of *FLCN* by DAVID tool.

Table 1. Gene enrichment analysis (BP)

Gene term	Gene count	Genes	P-value
GO:1904263–positive regulation of TORC1 signaling	11	<i>FLCN</i> , RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4, FNIP1, FNIP2	1.4459001395953574E-26
GO:0032008–positive regulation of TOR signaling	10	<i>FLCN</i> , RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9, LAMTOR2,LAMTOR1, LAMTOR4, FNIP1	1.6948069698738623E-24
GO:0071230–cellular response to amino acid stimulus	08	RRAGA, RRAGC, RRAGB, RRAGD, SLC38A9,LAMTOR2, LAMTOR1, LAMTOR4	3.19919449470896E-16
GO:0009267–cellular response to starvation	06	<i>FLCN</i> , RRAGA, RRAGC, RRAGB, RRAGD, FNIP1	1.9672145764390107E-10
GO:0008104–protein localization	06	RRAGA, RRAGC, RRAGB, RRAGD, LAMTOR2, LAMTOR1	6.407977009996166E-9

Table 2. Gene enrichment analysis (CC)

Gene term	Gene count	Genes	P-value
GO:1990877–FNIP-folliculin RagC/D GAP	08	<i>FLCN</i> , <i>RRAGA</i> , <i>RRAGC</i> , <i>SLC38A9</i> , <i>LAMTOR2</i> , <i>LAMTOR1</i> , <i>LAMTOR4</i> , <i>FNIP2</i>	4.187068176178242E-23
GO:0005765–lysosomal membrane	11	<i>FLCN</i> , <i>RRAGA</i> , <i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i> , <i>SLC38A9</i> , <i>LAMTOR2</i> , <i>LAMTOR1</i> , <i>LAMTOR4</i> , <i>FNIP1</i> , <i>FNIP2</i>	6.402440946010609E-18
GO:0005764–lysosome	08	<i>FLCN</i> , <i>RRAGA</i> , <i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i> , <i>SLC38A9</i> , <i>LAMTOR1</i> , <i>LAMTOR4</i>	1.752157273260692E-11
GO:1990131–Gtr1-Gtr2 GTPase complex	04	<i>RRAGA</i> , <i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i>	3.160221627082143E-10
GO:0071986–regulator complex	04	<i>SLC38A9</i> , <i>LAMTOR2</i> , <i>LAMTOR1</i> , <i>LAMTOR4</i>	1.5793162005874317E-9

Table 3. Gene enrichment analysis (MF)

Gene term	Gene count	Genes	P-value
GO:0051020–GTPase binding	04	<i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i> , <i>LAMTOR1</i>	9.791331088746019E-7
GO:0060090–molecular adaptor activity	05	<i>RRAGC</i> , <i>RRAGD</i> , <i>LAMTOR2</i> , <i>LAMTOR1</i> , <i>LAMTOR4</i>	1.7852304320294049E-6
GO:0043495–protein-membrane adaptor activity	03	<i>RRAGA</i> , <i>RRAGC</i> , <i>LAMTOR1</i>	1.343050898930897E-4
GO:0005085–guanyl-nucleotide exchange factor activity	04	<i>SLC38A9</i> , <i>LAMTOR2</i> , <i>LAMTOR1</i> , <i>LAMTOR4</i>	2.0318898449270994E-4
GO:0003924–GTPase activity	04	<i>RRAGA</i> , <i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i>	6.778171602462938E-4

Table 4. Gene enrichment analysis (KEGG)

Gene term	Gene count	Genes	P-value
hsa04150: mTOR signaling pathway	11	<i>FLCN</i> , <i>RRAGA</i> , <i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i> , <i>SLC38A9</i> , <i>LAMTOR2</i> , <i>LAMTOR1</i> , <i>LAMTOR4</i> , <i>FNIP1</i> , <i>FNIP2</i>	2.5004154144868723E-18
hsa04140: autophagy - animal	04	<i>RRAGA</i> , <i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i>	7.462133456980746E-4
hsa05131: shigellosis	04	<i>RRAGA</i> , <i>RRAGC</i> , <i>RRAGB</i> , <i>RRAGD</i>	0.002287907276650819

3.11. Correlation analysis

The Mann–Whitney U analysis was carried out to identify mutant genes correlated with *FLCN* expression. The study selected the top five mutant genes for LUAD, with $P < 0.05$ and $FC > 1.4$, by using the muTarget database. The top five mutant genes that positively correlated with the expression *FLCN* are PLA2G16, CARS2, SLCO4A1, SCN9A, and BRI3 in LUAD. Collectively, these results suggested that *FLCN* gene expression has a strong correlation with different mutant genes in LUAD (**Figure 11**).

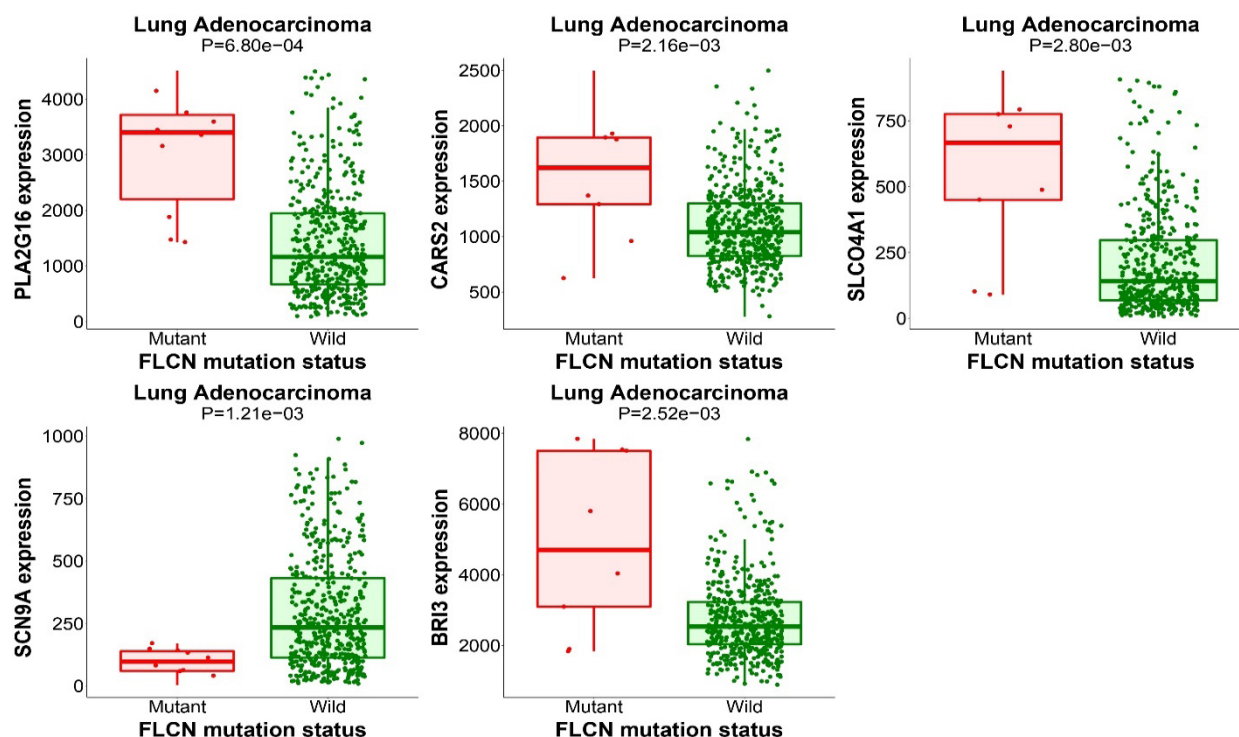


Figure 11. Correlation between *FLCN* expression in LUIAD.

4. Discussion

To conduct an assessment in LUAD, this study examined *FLCN* expression, prognosis, methylation, survival, mutations, and gene enrichment in this research article utilizing a variety of online bioinformatics tools. Furthermore, correlations between *FLCN* expression and crucial mutant genes also assessed. The results illustrated the importance of *FLCN* expression for human health as a putative regulator in the pathogenesis of LUAD and proposed a possible link between *FLCN* expression and the proliferation of LUAD tumors.

Globally, lung adenocarcinoma is the sixth most common cause of cancer-related fatalities. Most LUAD patients who have been diagnosed are in the middle and late stages, with many metastases, and have missed the best time to begin therapy because of the ambiguous early symptoms^[31]. The advancement of gene sequencing technologies in recent times has expanded the comprehension of the molecular pathogenesis of lung cancer. This enhances the overall survival of patients with advanced or metastatic diseases and encourages the discovery of new molecular markers and targeted therapies^[32]. A growing body of research has shown that hypoxia signaling modulates the development of LUAD, and genes associated with hypoxia may act as predictive indicators for individuals with LUAD. According to reports, hypoxia-stimulated GBE1 expression controls metabolic reprogramming, which aids in the progression of LUAD^[33]. The functions of hypoxia-related DNA methylation-driven genes in the development of LUAD are revealed by genome-wide analysis^[34]. The poor prognosis of LUAD and medication resistance are caused by hypoxia-induced cell stemness^[35].

The human folliculin (*FLCN*) gene encodes at least two major transcript variants on chromosome 17p11.2. Transcript variant 1 encodes the longer isoform that is 3,723 bp in length containing 14 exons of which 11 exons are coding. Transcript variant 2 is a shorter isoform containing 8 exons and uses an alternate splice site in the 3'

coding region to produce a distinctly different carboxy (C)-terminus compared to transcript variant 1. A 3.8 kb *FLCN* transcript was found by northern blot analysis in a wide range of adult tissues, including the brain, heart, placenta, testis, skin, lung, and kidney, as well as in the lung, liver, brain, and kidney of fetuses ^[16]. In BHD families and in families where spontaneous pneumothorax is the only symptom, more than 150 distinct mutations covering the whole *FLCN* coding area have been found and recorded in the FLCN Leiden Open Variation Database ^[36]. After discovering *FLCN* mutations in the hereditary kidney cancer form known as BHD syndrome, scientists looked into the possibility that *FLCN* mutations could also be the cause of random renal cancers that shared histological similarities with BHD-associated tumors. On the other hand, there was very little *FLCN* mutation frequency in sporadic kidney cancers ^[37]. There are now two documented naturally-occurring animal models of BHD, that support *FLCN* role as a tumor suppressor. The Nihon rat model of BHD, a type of Sprague-Dawley rat that spontaneously acquired a germline *FLCN* mutation, develops kidney tumors ^[38]. The initial indications of *FLCN* possible involvement in mTOR pathway modulation came from studies conducted in vivo in animals lacking in *FLCN*. Mice with *FLCN* inactivation directed towards the kidney distal nephron experienced polycystic kidneys and cystic renal disease before succumbing to renal failure at three weeks of age ^[39,40]. Studies in *FLCN* deficient *in vitro* and *in vivo* models provide further support for regulation of PPARGC1A by *FLCN*. A genetic analysis of the primary gene responsible for BHD syndrome, the *FLCN* gene, is crucial to the final diagnosis of BHD syndrome. More than 200 pathogenic or probably pathogenic *FLCN* variations are present in the HGMD database. The majority of *FLCN* variations, including nonsense, frameshift, and splice site variants, are truncating variants. Regarding *FLCN* c.1432 + 1G > A, which is also referred to as IVS12 + 1G > A, separate investigations have identified this classical splicing site mutation in individuals with BHD syndrome ^[23].

The current assessment used the UALCAN database to find *FLCN* expression in LUAD. Studies have indicated that the expression of *FLCN* is elevated in a variety of cancer stages, types of cancer development, age, gender, and racial groups. The primary objective of the flow outcome is to show that LUAD tissues exhibited notably greater levels of *FLCN* expression than normal control samples in relation to the progression of the tumor. The STRING and DAVID tools research also demonstrated the diversity of the *FLCN* gene and how it interacts with other genes to be a crucial part of several biological pathways and activities. Additionally, muTarget online tool was used to assess the correlation between *FLCN* expression and crucial mutant genes. The results indicated that the degree of *FLCN* expression in LUAD was a different unfavorable prognostic factor. Subsequent analyses ought to focus on the prognostic significance of *FLCN* expression at different stages of cancer development.

5. Conclusion

In this study, several bioinformatics-based online databases were used to thoroughly examine *FLCN* in LUAD cancer type. According to this study, there was a significant increase in *FLCN* expression, which was linked to aggressive clinicopathological aspects of LUAD, as well as survival duration and metastasis. When considered collectively, these findings demonstrated that *FLCN* has carcinogenic functions and may be a viable target for treatment in individuals suffering from LUAD.

Disclosure statement

The authors declare no conflict of interest.

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IGF2BP1 in Mesenchymal GBM Immune Signalling Regulation via c-Myc

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Abstract: Glioblastoma multiforme (GBM) is a highly aggressive and lethal brain tumor, with poor patient prognosis and median overall survival of only 10 months despite the current Stupp protocol treatment, due to its high aggressiveness and recurrence rate. This study investigated the role of the IGF2BP1 gene in mesenchymal glioblastoma multiforme (GBM), revealing that IGF2BP1 is upregulated in tumor tissues compared to adjacent normal tissues and positively correlates with MYC gene expression and poor patient prognosis. Immune infiltration analysis showed that IGF2BP1 is associated with specific immune cell populations, and GSVA analysis confirmed its positive correlation with the immune functions of most B cells and macrophages. The mechanism of IGF2BP1 regulating c-Myc expression in mesenchymal GBM and its subsequent impact on immune-related signalling pathways, thereby affecting the immune microenvironment of tumors and patient prognosis, provides new targets and ideas for future immunotherapy of mesenchymal GBM.

Keywords: Glioblastoma multiforme; Immune; IGF2BP1; c-Myc

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1. Introduction

Glioma primarily originates from glial cells within the brain represent the most common type of tumor in the central nervous system (CNS), accounting for 30% of all primary brain tumors and 80% of all malignant brain tumors ^[1]. According to the World Health Organization (WHO) classification, grade 4 glioma, known as glioblastoma (GBM), comprises approximately 57% of all gliomas, with an average annual incidence rate of 4.23 per 100,000 individuals ^[2]. GBM is characterized by its highly aggressive nature and a markedly high mortality rate, evidenced by a 5-year survival rate of merely 6.8% post-diagnosis. Its development is associated with various factors, including environmental influences, genetic variations, and inherited syndromes ^[1-3].

Currently, the Stupp standard plan is a widely adopted clinical treatment plan for GBM, which is to surgically remove the tumor within the maximum safety range, followed by temozolomide concurrent chemotherapy and radiotherapy, and then temozolomide maintenance chemotherapy starting four weeks after the end of

radiotherapy. However, the prognosis for patients remains poor, with a median overall survival (OS) of merely 10 months^[4–7]. Additionally, targeted treatment methods based on specific genetic mutations or abnormalities in signaling pathways, including the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) and the p53, retinoblastoma (RB), and epidermal growth factor receptor (EGFR) pathways^[8]. The failure of targeted drugs in the later stages of clinical development suggests that most GBM multiforme is not even close to being a single-pathway-driven disease that can be treated with targeted therapy. In addition, the latest immunotherapy chimeric antigen receptor (CAR)-T cell therapy has not demonstrated sustained antitumor activity against GBM multiforme^[9]. In addition to the limitations of CAR-T cells in their ability to migrate and infiltrate solid tumors, the antitumor cytotoxicity of CAR-T cells is also constrained by the immunosuppressive TME containing immunosuppressive cells and immune checkpoint molecules that promote immune escape^[10]. Therefore, studying the interaction between GBM tumor cells and immune cells, especially exploring the mechanisms by which tumor cells secrete factors that actively promote tumor cell proliferation and invasion and act on immune cells to form a tumor immunosuppressive microenvironment in the TME, can guide the development of new immune interventions and improve the prognosis of patients with GBM.

GBM microenvironment encompasses vascular compartments and immune cells such as monocytes, macrophages, microglial compartments, or T cells^[8,11,12]. GBM promotes tumor growth, proliferation, and invasion by altering the interaction of immune cells and modulating the mutual regulation of immune molecules, thereby influencing both innate and acquired immunity through mechanisms of immune evasion. It has been demonstrated that the depletion of glioma-associated microglia/macrophages (GAMs) can significantly reduce tumor growth^[13,14]. Compared with traditional treatment, immunotherapy, such as immune checkpoint inhibitors and tumor vaccines-targets specific tumor cells without damaging normal cells, potentially reducing side effects^[15–17]. Moreover, by activating the patient's immune system to combat the tumor, immunotherapy can provide more durable effects, continuously monitoring and eliminating residual tumor cells to prevent recurrence^[16,17]. However, the efficacy of these treatments varies among GBM patients due to differences in individual conditions, tumor types, immune statuses, and other factors.

In the innate immune response, GBM cells release cytokines such as TGF- β , IL-10, IL-4, and IL-13, promoting the acquisition of an M2 macrophage-like phenotype by microglia, associated with increased invasiveness and poor prognosis^[18]. Additionally, GBM stem cells (GSC) can recruit tumor-associated macrophages (TAM) by secreting cytokines related to selective activation like osteopontin, IL-6, IL-8, and TNF- α . TAM further enhances the growth, invasion, and angiogenesis of GBM by secreting various growth factors and inflammatory factors such as TGF- β 1, VEGF, epidermal growth factor (EGF), and IL-10^[19–22]. Furthermore, the overexpression of oncogenes and loss of tumor suppressor genes can diminish the sensitivity of cells to immune-mediated killing, facilitating immune evasion in GBM^[23].

GBM cells can suppress the cellular immune activity of the adaptive immune system within the GBM microenvironment. GBM-associated myeloid-derived suppressor cells (MDSCs) have the potential to promote the suppressive activity of regulatory B (Bregs) cells towards CD8 T cell activation and acquisition of effector phenotypes by delivering membrane-bound PD-L1-containing microvesicles^[24]. Additionally, the TGF- β released by GBM cells can suppress inflammation and promote immunosuppression by inhibiting T cell proliferation and inducing T cell apoptosis^[25,26]. Meanwhile, GBM cells can release cytokines such as IL-10, which participate in the upregulation of signal transducer and activator of transcription 3 (STAT3), further suppressing the activity of immune cells^[26].

There are differences in the prognosis and survival rates of patients with different GBM subtypes. Based on differences in gene expression, GBM is classified into four subtypes: classical, mesenchymal, neural, and proneural. The mesenchymal subtype, characterized by its high invasiveness and angiogenesis, poses significant challenges for traditional treatment methods, leading to poor patient prognosis due to high recurrence rates and short survival times^[27,28]. Additionally, this subtype possesses resistance to apoptosis, further increasing its malignancy and treatment difficulty^[29]. Due to the high invasiveness and angiogenesis of mesenchymal GBM, traditional surgical resection, radiation therapy, and chemotherapy often find it difficult to eliminate the tumor. Even with intensive treatment, the prognosis for patients is relatively poor, with a high recurrence rate and short survival time^[30]. Compared to the other three subtypes, mesenchymal GBM has the strongest immunogenicity^[31]. It not only exhibits a high ratio of macrophages/microglia and an abundance of neutrophils, but also a low abundance of NK cells^[30]. Additionally, it can activate M2-type macrophages and suppress T cell activity, thereby mediating immune suppressive TAM^[32]. Increased mRNA expression of various cytokines, immune cell markers, and immune-related signaling pathways makes this subtype the most immunologically and inflammatory-associated subtype^[33,34]. The immune evasion characteristics of mesenchymal GBM suggest that immunotherapy may be more effective in stimulating immune responses, thereby contributing to the elimination of tumor cells and improving patient prognosis.

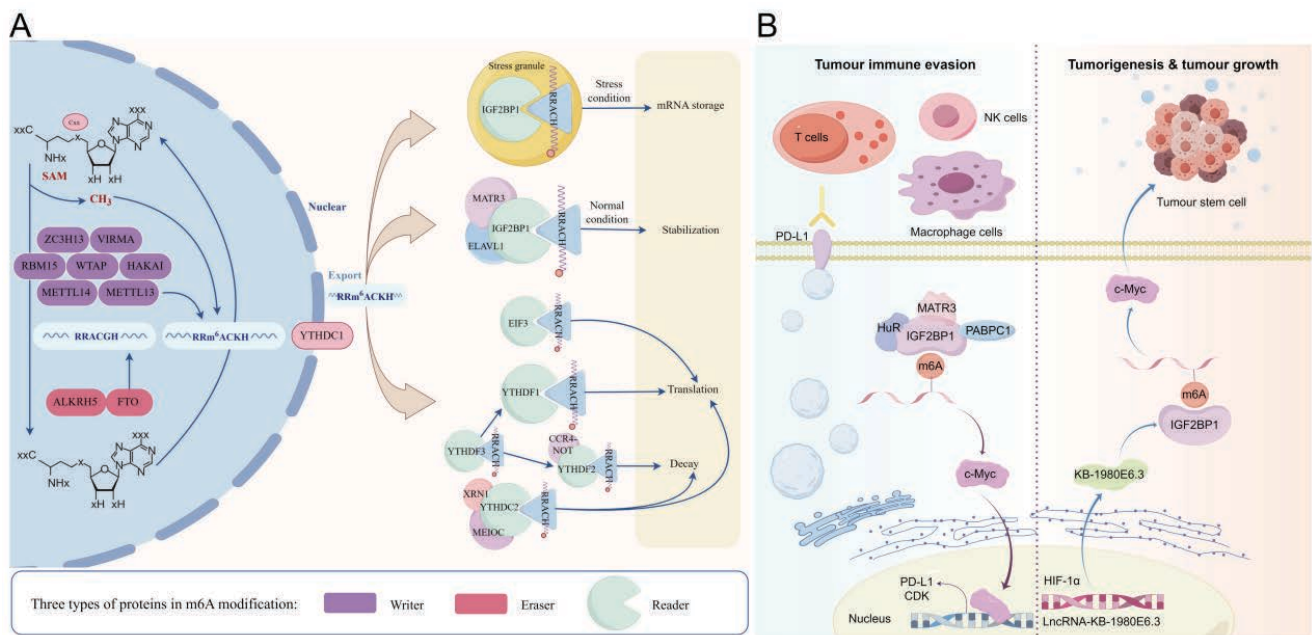


Figure 1. (A) The three classes of proteins—Writers, Erasers, and Readers—dynamically regulate m6A modification flexibly. (B) IGF2BP1 affects the TME and the tumor itself by regulating the expression of c-Myc in other tumors.

As the most abundant modification in eukaryotic mRNAs, m6A is considered the most common, frequent, and conserved internal modification in cancer development^[35]. This dynamic regulation is facilitated by three classes of proteins: “writers,” “erasers,” and “readers” (**Figure 1A**)^[36]. “Writer” proteins, such as METTL3 and METTL14, collaborate with the RNA-binding protein WTAP to add m6A modifications to mRNAs^[37]. “Eraser” proteins, including FTO and ALKBH5, remove m6A modifications, affecting mRNA stability and translation efficiency^[36]. “Readers” such as YTHDF1/2/3, YTHDC2, and IGF2BP1/2/3, recognize and bind to m6A-modified transcripts, influencing gene expression through processes such as mRNA stability, mRNA splicing, and

translation efficiency^[36]. Studying the role of m6A RNA modification in GBM has revealed that this modification affects immune responses by regulating the expression of specific genes and intercellular interactions, with reading proteins such as IGF2BP1 playing a crucial role in GBM^[38–42]. The upregulation of this protein can regulate the proliferation and survival of GBM cells, modulate the stability and expression of specific mRNAs such as c-Myc, significantly reduce the expression of PD-L1 in the tumor, and consequently promote immune evasion (Immune cells such as CD4, CD8 T cells, CD56 NK cells, and F4/80 macrophages), ultimately leading to tumor progression^[38–41]. As a key regulator in the TME, c-Myc promotes the expression of CD47 and PD-L1 by binding to their gene promoters, with PD-L1 expressed on cells such as T cells, B cells, and macrophages, thereby not only inducing exhaustion and apoptosis in CD8 T cells but also suppressing their proliferation and cytotoxicity through binding to PD-1 on their surface^[42,43] (**Figure 1B**). Additionally, the upregulation of c-Myc expression not only triggers the expression of cytokines such as IL-4, IL-10, and TGF- β , resulting in the polarization of macrophages towards the M2 phenotype^[44], but also prompts B cells to facilitate T cell apoptosis via the expression of PD-L1 and IL-10, and secrete TGF- β to impede the function of NK cells^[45], thereby further contributing to immune evasion. Research has found that this gene can also maintain the stem cell properties of tumors and directly promote tumor growth through the LncRNA KB-1980E6.3/IGF2BP1/c-Myc pathway^[42] (**Figure 1B**). The immunosuppressive TME formed by various factors, as well as their direct impact on the tumor, lead to the occurrence, migration, and metastasis of the tumor^[40–42,46,47].

2. Materials and methods

In this study, a comprehensive approach was employed to investigate the role of the IGF2BP1 gene in mesenchymal glioblastoma (GBM) by utilizing R (2022.02.3 Build 492) and TIMER2.0 (<http://timer.cistrome.org/>) to analyze gene expression differences between tumor and adjacent normal tissues in GBM from TCGA, conducting statistical tests to compare patient characteristics and outcomes, employing the CIBERSORT algorithm for immune infiltration analysis, performing gene set variation analysis (GSVA) to assess immune process enrichment, and conducting cell culture and coculture experiments using a Transwell system to measure cytokine levels, glioma cell viability, and apoptosis rate in U87 glioma cells cocultured with induced M2 macrophages.

3. Results

Utilizing TIMER2.0 (<http://timer.cistrome.org/>) to analyze the expression difference of the IGF2BP1 gene between tumor and adjacent normal tissues in all tumors from TCGA. In GBM, the expression of the IGF2BP1 gene in tumor tissues was significantly higher than that in adjacent normal tissues ($P = 0.0087$) (**Figure 2A**).

Further analysis was conducted on the mesenchymal GBM in the TCGA database using the relevant genes for GBM subtype classification from the study by Chanoch-Myers *et al.*^[32]. The results showed that patients without MGMT promoter methylation were older ($P = 0.024$) (**Figure 2B**); there was a positive correlation between IGF2BP1 expression and MYC gene expression ($P = 0.005$) (**Figure 2C**); patients with low IGF2BP1 expression had significantly better OS compared to those with high IGF2BP1 expression ($P = 0.036$) (**Figure 2B** and **Figure 2D**); using the CIBERSORT algorithm for immune infiltration analysis of these samples, the expression of “B cells native” and “Macrophage M0” are positively correlated with the expression of the IGF2BP1 gene ($P = 0.0073$, $P = 0.0395$) (**Figure 2E**), while the expression of “B cells memory” and “Macrophage M2” are negatively correlated with the expression of the IGF2BP1 gene ($P = 0.0108$, $P = 0.0048$) (**Figure 2E**).

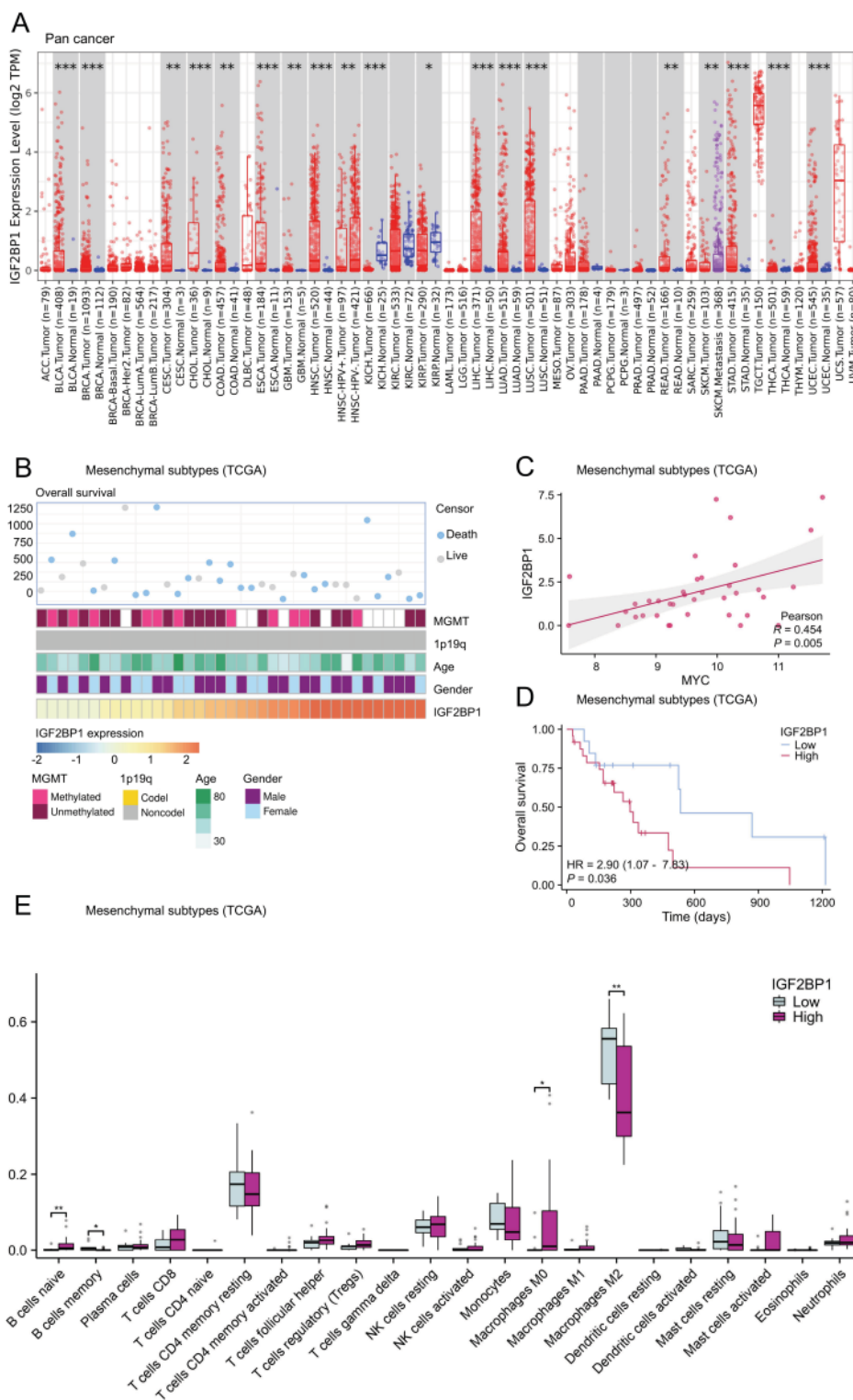


Figure 2. (A) Expression profile of IGF2BP1 across multiple cancer types; (B) Landscape of IGF2BP1-related clinicopathological features in the mesenchymal GBM from the Cancer Genome Atlas (TCGA) database; (C) Correlation between IGF2BP1 gene expression and MYC gene expression in the mesenchymal GBM from the TCGA database; (D) Prognostic analysis of IGF2BP1 gene expression in the mesenchymal GBM from the TCGA database; (E) Association between IGF2BP1 expression and immune cell infiltration in the mesenchymal GBM from the TCGA database.

Gene set variation analysis (GSVA) was performed on the immune cells related to this subtype to determine the enrichment scores for immune processes (**Figure 3**). Correlation analysis between the enrichment scores and IGF2BP1 expression revealed a positive correlation between IGF2BP1 expression and the immune functions of most B cells and macrophages.

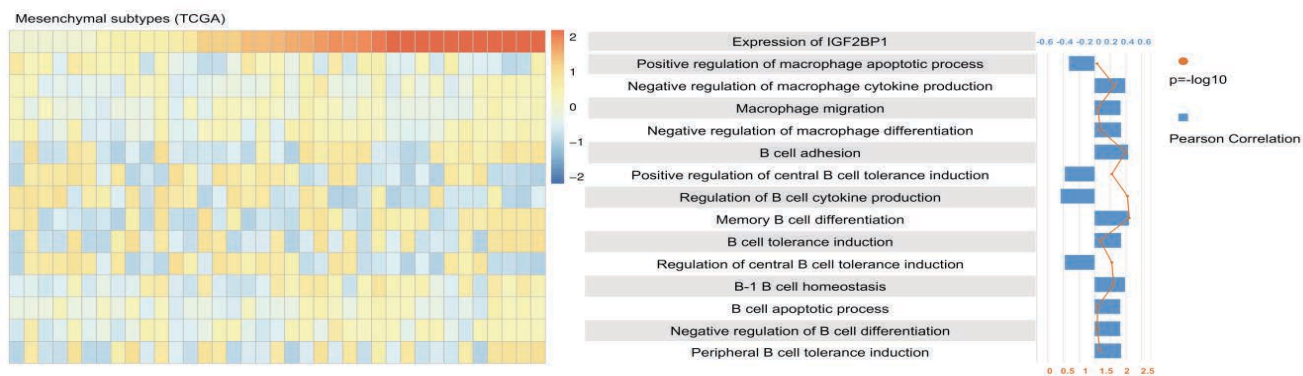


Figure 3. Gene set variation analysis of the mesenchymal GBM from the TCGA database.

4. Conclusion

Glioma, particularly GBM, is one of the most common malignancies of the Central Nervous System (CNS). Its highly aggressive nature and extremely high mortality rate have posed significant challenges in clinical treatment. The analysis of TCGA data revealed that IGF2BP1 expression is significantly elevated in glioblastoma (GBM) tumor tissues compared to adjacent normal tissues. Further investigation in the mesenchymal GBM subtype identified that patients without MGMT promoter methylation were older and that IGF2BP1 expression positively correlated with MYC gene expression. Notably, patients with low IGF2BP1 expression exhibited better overall survival (OS) than those with high expression. Immune infiltration analysis showed that IGF2BP1 expression positively correlated with “B cells native” and “Macrophage M0” but negatively with “B cells memory” and “Macrophage M2.” GSVA indicated a positive correlation between IGF2BP1 expression and the immune functions of most B cells and macrophages.

Current treatment methods are limited, and the immunoregulatory mechanisms within the Tumor Microenvironment (TME) are complex. The focus on the role of m6A RNA modification in GBM immune evasion may provide new insights for future immunotherapy approaches.

Disclosure statement

The authors declare no conflict of interest.

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The Impact of Narrative Nursing on Body Image, Disability Acceptance, and Psychological Hope Level in Young and Middle-aged Breast Cancer Patients After Surgery

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Abstract: *Objective:* To investigate the effects of narrative nursing on body image, disability acceptance and psychological hope level of young and middle-aged breast cancer patients after radical surgery. *Methods:* A non-randomized quasi-experimental study was used to divide 80 patients in the Department of Breast Surgery of a tertiary cancer hospital in Yunnan Province from October 2023 to September 2024 as the study subjects and the study subjects were divided into the intervention group and control group with 40 cases in each group. The intervention was performed within 1 month after surgery, and a total of 5 interventions were performed. The two groups of patients were evaluated by the breast cancer patients' body image self-rating questionnaire, the revised version of the Disability Acceptance Scale, and the Herth Hope Assessment Scale. *Results:* There were no significant differences in BISQ-BC, ADS-R, and HHI between the intervention group and the control group before intervention ($P > 0.05$). After the intervention, the BISQ-BC score of the intervention group was lower than that of the control group, and the ADS-R score and HHI score were higher than those of the control group, and the difference was statistically significant ($P < 0.05$). *Conclusion:* Narrative nursing can effectively improve the physical image level of young and middle-aged breast cancer patients, improve the disability acceptance and psychological hope level of young and middle-aged breast cancer patients, and help them vent their negative emotions, gain confidence in life, and improve their quality of life.

Keywords: Narrative nursing; Breast cancer; Radical surgery; Body image; Disability acceptance

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1. Introduction

Breast cancer is the most common malignant tumor among women worldwide, with a peak incidence age of 45 to 54 years in China, and a relatively high proportion of young and middle-aged patients, showing a trend towards younger ages ^[1,2]. According to the Cancer Society data report ^[3], breast cancer ranks first with approximately 2.26 million new cases. Currently, the treatment of breast cancer is primarily surgical, with chemotherapy and radiotherapy as adjuncts. The overall treatment course is long, significantly affecting the patient's physical and mental health. Studies have shown that breast cancer patients experience psychological issues such as anxiety, depression ^[4], body image distress ^[4], and low mental health levels ^[5], with 50% to 67% of breast cancer patients experiencing body image disorders ^[6], which may persist. A good level of body image can help patients better cope with cancer, while a low level of body image can affect the physiological and psychological functions of breast cancer patients and their partner relationships, impacting their sexual life and marital quality ^[7], leading to social avoidance behaviors and psychological distress ^[8], significantly affecting the quality of life ^[9]. Therefore, early assessment of body image and the initiation of positive psychological interventions are crucial for helping young and middle-aged breast cancer patients post-surgery to accept themselves, improve their disability acceptance levels, and enhance their post-surgery quality of life. Narrative nursing refers to the practice where nurses listen to patients' personal experiences, absorb the essence of their stories, help patients reconstruct their life patterns, and then identify personalized medical and nursing points, implementing medical and nursing interventions for patients ^[10]. Currently, narrative nursing is mainly used in areas such as malignant tumors ^[11], stroke ^[12], and nursing education ^[13], but research on improving the body image levels of young and middle-aged breast cancer patients post-surgery is still relatively scarce. Based on this, this study applies narrative nursing to intervene in young and middle-aged breast cancer patients post-surgery, exploring its effects on improving body image levels, disability acceptance, and psychological states of these patients, with the aim of providing relevant reference data.

2. Objectives and methods

2.1. Study subjects

This study is quasi-experimental research, using a convenience sampling method to select 80 postoperative breast cancer patients who visited a hospital in Yunnan. The province from October 2023 to September 2024 as the study subjects. The 80 subjects were divided into groups according to different wards, with 40 cases in each group. Interventions were carried out within one month after surgery, a total of 5 times.

Inclusion criteria: (1) Age ≥ 18 years; (2) Diagnosed with breast cancer by pathological diagnosis and underwent a radical surgical plan; (3) Body image score ≥ 70 points; (4) Conscious, with basic reading and understanding ability; (5) Agreed to participate in this study.

Exclusion criteria: (1) Those who underwent breast reconstruction; (2) With other malignant tumors; (3) History of mental illness in the past. The study was approved by the hospital ethics committee (KYLX2023-181). There was no statistically significant difference in the general data of the two groups of patients ($P > 0.05$) (**Table 1**).

Table 1. General information of the two groups ($n = 80$)

Project	Classification	Control group		Intervention group		Statistical value	<i>P</i>
		Mean ± SD / <i>n</i> (%)					
Age		46.8 ± 6.80		47.55 ± 5.66		3.429 ^a	0.593
Nation	Han Chinese	37	92.5	29	72.5	5.541 ^c	0.037
	Other ethnic	3	7.5	11	27.5		
Religious beliefs	Yes	2	5.0	1	2.5	0.346 ^c	1.000
	No	38	95.0	39	97.5		
Current residence	City	27	67.5	36	90.0	4.731 ^b	0.052
	Rural	13	32.5	4	10.0		
Educational level	Elementary school and below	12	30.0	11	27.5	4.675 ^b	0.200
	Middle school	10	25.0	8	20.0		
	High school / Secondary vocational school / Vocational high school	11	27.5	6	15.0		
	Bachelor's degree or above	7	17.5	15	37.5		
Marital status	Married	35	87.5	39	97.5	4.216 ^c	0.121
	Unmarried	0	0	0	0		
	Divorced	4	10.0	0	0		
	Widowed	1	2.5	1	2.5		
Occupational status	In service	11	27.5	15	37.5	1.001 ^b	0.606
	Retirement	8	20.0	6	15.0		
	Unemployed	21	52.5	19	47.5		
Monthly per capita income	≤ 3000	7	17.5	7	17.5	0.971 ^b	0.606
	3001-5000	18	45.0	14	35.0		
	≥ 5000	15	37.5	19	47.5		
Health insurance method	Urban and Rural Resident Medical Insurance	27	67.5	23	57.5	5.556 ^c	0.135
	Employee Medical Insurance	13	32.5	17	42.5		
Disease staging	I	0	0	7	17.5	7.702 ^c	0.096
	II	31	77.5	25	62.5		
	III	9	12.5	8	20.0		
Surgical methods	Radical improvement surgery	30	75.0	22	55.0	3.516 ^c	0.061
	Mastectomy	10	25.0	18	45.0		

2.2. Intervention methods

2.2.1. Control group

Patients receive routine breast cancer specialized care, which includes health education and psychological counseling related to breast malignancy surgery and chemotherapy, conducted through a combination of oral and health education manual methods.

2.2.2. Intervention group

Based on the control group, narrative nursing is carried out. Nurses are required to actively listen to patients' stories throughout the work process and identify nursing points based on the content, reconstructing the meaning of the stories to effectively control patients' adverse psychological states ^[14].

(1) Formation of the narrative nursing intervention team

The narrative team consists of one national second-level psychological consultant and one medical social worker, responsible for theoretical and practical training; two deputy directors of breast surgery, responsible for formulating cancer treatment plans for patients under their care and assessing changes in their conditions, grasping key points of treatment; one master's supervisor, responsible for overseeing the implementation of the narrative nursing plan; one head nurse of breast surgery, responsible for coordinating the shifts of the narrative team members and organizing training and assessment for the narrative nurses; one graduate student who has undergone narrative training, who implements narrative nursing interventions based on the specific conditions of patients; and two duty nurses, who assist with routine care for both the control and intervention groups.

(2) Formulation of the narrative nursing intervention plan

The researcher reviews domestic and international literature related to "narrative nursing" and "body image," summarizes research progress, and formulates a plan based on the actual situation and nursing characteristics of the department.

- (a) Implementation personnel: Each narrative nursing intervention is carried out by two nurses working together, with the narrative nursing personnel being the patient's fixed duty nurse (i.e., the narrative nurse in the narrative research group), facilitating trust from the patient;
- (b) Implementation method: Conducted in the form of one-on-one, face-to-face interviews;
- (c) Intervention time: Interventions are carried out twice, once before the patient's radical surgery, three days after surgery, before discharge, during the home period, and one month after surgery, with each session expected to last 20–30 minutes.
- (d) Implementation location: The department interview room.

(3) Implementation of the narrative nursing plan

- (a) Establishing trust relationships: After the patient is admitted, the patient is fully understood and recognized, and psychological counseling is provided. Guide the patient to speak freely and express themselves, and in communication, respond with smiles and nods without interrupting the patient, listen carefully and patiently to the patient's stories, and make positive and appropriate responses to the patient's stories, respecting the patient's privacy during communication. Establish a trusting relationship with the patient, allowing the patient to open up and share. Focus on understanding the patient's psychological state, personality traits, job nature, family background, and family relationships.
- (b) External deconstruction of problems: Uncover meaningful and positive related events, discover the patient's strengths, improve the patient's negative attitude, adjust the patient's self-body image cognition, and help the patient face the disease positively. Problem externalization: Guide the patient to name the body image problems that trouble them, assist the patient in separating self-identity from physical appearance, and explain that the problem is not their problem, concretizing the problem, increasing the patient's sense of control over the problem, and focusing energy on solving the problem. Deconstructing the problem: Reconstructing self-identity. Through the patient's responses, dig out the underlying social

and cultural factors related to the patient's generation of this cognition and ideas, explain the root causes of the problem from the perspective of the cultural background in which the patient is situated, separate the patient from the problem, and view the problem from an objective perspective.

- (c) Problem rewriting, external witnesses: Transfer the patient's positive self-evaluation to existing problems, and guide the patient to make decisions for themselves. During conversations with patients, if a deviation between the nurse's understanding and the patient's perception is detected, immediate self-reflection is made; and together with the patient, explore exceptional events, mainly exploring those successful, proud, beautiful, positive things in the patient's growth process, especially exploring the exceptional events where the patient overcomes internal feelings of disease stigma and recognizes their value.
- (d) Treatment documents: The form of treatment documents is diverse, not limited to format, such as certificates, awards, messages, letters, etc., tailored to the individual. Using a sense of ritual to strengthen the patient's beliefs, and providing psychological support when the patient wants to give up. The above stages cover the entire narrative nursing interview process, with no clear chronological order, and the specific practical methods vary according to the nurse's communication with different patients.

2.3. Research tools

2.3.1. General information questionnaire

Based on the review of relevant literature and consultation with experts, a summary of general demographic information was made. This includes: age, ethnicity, religious beliefs, current residence, educational level, marital status, employment status, average monthly household income, medical insurance method, disease stage and grade, and surgical method.

2.3.2. Breast Cancer Patient Body Image Self-Rating Questionnaire (BISQ-BC)

Developed by Zhou *et al.* (2018) ^[15] based on the multidimensional theory of body image, it consists of 5 dimensions and 26 items. The 5 dimensions are: the social change dimension, behavioral change dimension, activity change dimension, role change dimension and psychological change dimension. The questionnaire uses a Likert 5-point scale, with a total score ranging from 26 to 130, where a higher score indicates more severe body image disturbance. The Cronbach's α coefficient of the scale is 0.90 (Appendix C).

2.3.3. Acceptance of Disability Scale-Revised (ADS-R)

This questionnaire was developed by Groomes *et al.* (2007) ^[16] and translated by Alvarez-Pardo *et al.* (2023) ^[17]. The scale consists of 32 items, using a Likert 4-point scale, with a total score ranging from 32 to 128. Scores from 32 to 64 indicate a low level, 65 to 96 indicate a moderate level, and 97 to 128 indicate a high level. The scale includes four dimensions: inclusion dimension (inclusion of the impact of breast loss), transition dimension (shift from comparative value to intrinsic value), expansion dimension (expansion of the value range), and affiliation dimension (affiliation to body image). The correlation coefficients between each dimension and the total scale range from 0.609 to 0.890.

2.3.4. Herth Hope Index (HHI)

Developed by the American scholar Herth (1992) ^[18] and translated and introduced into China by Zhao *et al.* (2000) ^[19] from China Medical University in 1999. The scale contains 3 dimensions, with a total of 12 items.

These include: positive attitude (items 1, 2, 6, 11), positive action (items 4, 7, 10, 12), and intimate relationships (items 3, 5, 8, 9). It uses a Likert 4-point scale, with a total score ranging from 12 to 48, where a higher score indicates a higher level of hope. The Cronbach's α of the scale is 0.87, and the test-retest reliability is 0.92.

2.4. Quality control

- (1) Preparation stage: Narrative nurses receive narrative nursing training and assessment before starting narrative nursing work to ensure the effectiveness and homogeneity of narrative nursing;
- (2) Data collection stage: Investigators are required to fully understand the questionnaire and distribute it using a unified standard instruction, with timely and deletion of questionnaires with regular answers and a missing rate of more than 15%;
- (3) Entry stage: Double entry of results is implemented using EpiData 3.1 software, and inconsistencies are corrected by referring to the original patient questionnaires.

2.5. Statistical analysis

SPSS 27.0 statistical software is used for data analysis. Measurement data that conform to the normal distribution are expressed as mean \pm standard deviation (SD), and count data are expressed as frequency and percentage. Comparisons between groups and within groups are made using two independent sample *t*-tests and paired *t*-tests. A *P*-value of less than 0.05 is considered statistically significant.

3. Results

3.1. Comparison of BISQ-BC scores for body image between two groups of patients

After intervention, the BISQ-BC scores of the intervention group patients decreased compared to before intervention, and the BISQ-BC scores of the intervention group patients were lower than those of the control group, with a statistically significant difference ($P < 0.05$) (Table 2).

Table 2. Intra-group comparison of body image (BISQ-BC) scores before and after intervention between the two groups (points, mean \pm SD)

Project	Classification	Before intervention ($n = 40$)	After intervention ($n = 40$)	<i>t</i>	<i>P</i>
BISQ-BC	Control group	105.90 \pm 5.07	104.50 \pm 5.22	1.329	0.192
	Intervention group	104.33 \pm 7.76	60.98 \pm 7.68	23.089	< 0.001
Related psychological changes	Control group	33.08 \pm 3.03	32.75 \pm 3.06	0.483	0.632
	Intervention group	33.18 \pm 3.69	17.18 \pm 3.46	19.634	< 0.001
Related behavior change	Control group	29.45 \pm 2.22	29.23 \pm 2.79	0.411	0.683
	Intervention group	28.78 \pm 3.93	19.95 \pm 3.84	10.080	< 0.001
Related role changes	Control group	22.25 \pm 3.28	22.08 \pm 2.14	0.291	0.711
	Intervention group	21.33 \pm 2.60	12.78 \pm 3.76	12.483	< 0.001
Behavioral change related to the subject	Control group	13.68 \pm 2.30	13.35 \pm 1.70	0.780	0.744
	Intervention group	13.20 \pm 2.77	6.95 \pm 2.36	10.493	< 0.001
Related social changes	Control group	7.45 \pm 0.96	7.10 \pm 1.53	1.595	0.119
	Intervention group	7.85 \pm 1.21	4.13 \pm 1.87	9.618	< 0.001

3.2. Comparison of ADSR scores before and after intervention in two groups of patients

After intervention, the ADSR scores of both groups of patients increased compared to before intervention, and the ADSR scores of the intervention group were higher than those of the control group, with a statistically significant difference ($P < 0.05$) (Table 3).

Table 3. Intra-group comparison of ADSR scores before and after intervention in two groups (points, mean \pm SD)

Project	Classification	Before intervention ($n = 40$)	After intervention ($n = 40$)	t	P
ADS-R	Control group	79.33 \pm 8.51	92.10 \pm 4.91	-7.680	< 0.001
	Intervention group	77.58 \pm 8.39	101.98 \pm 5.23	-15.598	< 0.001
Expand dimensions	Control group	26.38 \pm 2.43	28.23 \pm 2.84	-6.037	< 0.001
	Intervention group	27.20 \pm 2.35	30.85 \pm 2.55	-6.803	< 0.001
Inclusive dimension	Control group	20.68 \pm 3.93	25.28 \pm 2.30	-0.841	0.406
	Intervention group	19.85 \pm 4.51	29.15 \pm 2.32	-11.206	< 0.001
Transform dimensions	Control group	20.75 \pm 3.54	25.65 \pm 2.80	-0.548	0.587
	Intervention group	19.93 \pm 4.70	27.85 \pm 2.08	-1.216	< 0.001
Subordinate dimension	Control group	11.53 \pm 1.99	12.95 \pm 1.63	1.429	0.161
	Intervention group	10.60 \pm 2.31	14.13 \pm 2.51	-6.794	< 0.001

3.3. Comparison of HHI scores between two groups of patients before and after intervention

After intervention, the HHI scores of both groups of patients increased compared to before intervention, and the HHI scores of the intervention group were higher than those of the control group, with a statistically significant difference ($P < 0.05$) (Table 4).

Table 4. Intra-group comparison of Herth Hope Index (HHI) scores before and after intervention in two groups (points, mean \pm SD)

Project	Classification	Before intervention ($n = 40$)	After intervention ($n = 40$)	t	P
Herth	Control group	33.40 \pm 2.74	33.85 \pm 3.62	-0.845	0.403
	Intervention group	33.85 \pm 4.45	43.18 \pm 2.49	-11.031	< 0.001
Positive attitude	Control group	10.35 \pm 1.72	10.03 \pm 1.64	1.428	0.161
	Intervention group	10.70 \pm 2.13	14.23 \pm 2.49	-8.602	< 0.001
Positive action	Control group	11.75 \pm 0.93	12.03 \pm 1.29	-1.317	0.195
	Intervention group	12.18 \pm 1.30	14.58 \pm 1.24	-9.055	< 0.001
Intimate relationships	Control group	11.30 \pm 1.36	11.80 \pm 1.74	-1.612	0.115
	Intervention group	10.98 \pm 2.03	14.38 \pm 1.06	-9.365	< 0.001

4. Discussion

4.1. Narrative nursing can effectively improve patients' body image levels

Narrative nursing refers to patients narrating their experiences and stories, with nurses helping them to reconstruct

the meaning of their illness and life stories through their narratives. In the process of narration, nurses experience the patient's mental state, physical manifestations, and psychological changes on multiple levels, to achieve holistic nursing. The purpose of narrative nursing interventions is to enhance patients' self-awareness, analyze and affirm their positive strengths, and recognize the efforts patients make in overcoming difficulties, psychological issues, facing and conquering illness; through storytelling and conversation, nurses uncover potential psychological issues, provide positive guidance, and stimulate the patient's inner strength. In the narrative process, the patient is the protagonist in solving problems, while the nurse is a collaborator and guide^[20]. Nurses, as listeners, get close to the patient's life story, focusing on the internalized problems of the patient and externalizing them, transforming the patient's anxiety and unease into something objective, enhancing the courage to face problems; additionally, by helping patients find overlooked highlights and the positive forces behind them, patients are made to realize that they have the resources and abilities to solve problems, their potential and positive traits, and provide new choices, making them aware of the possibility of a new life, thus building confidence, seeing hope, and changing the patient's actions and self-identity^[21]. The results of this study show that after the intervention, the body image scores of the intervention group were significantly lower than those of the control group, consistent with related research findings^[22].

4.2. Narrative nursing can effectively improve patients' disability acceptance and psychological hope levels

The results of this study show that after implementing narrative nursing, the ADSR scores and psychological hope levels of the intervention group were higher than those of the control group ($P < 0.05$), indicating that narrative nursing can better improve patients' ADSR and psychological hope levels. The reason may be that narrative nursing combines humanities with medical care. When patients feel understood, they experience a profound sense of satisfaction, which also has a multiplier effect on improving patients' disability acceptance. Narrative nursing improves the confidence of patients with breast absence after radical breast cancer surgery through reasonable interventions, reducing the patient's sense of stigma. Narrative nursing can guide patients to open up channels for expressing negative emotions through storytelling, reducing the patient's self-imposed emotional burden, and allowing patients to actively explain and decompose the huge stressor of "cancer diagnosis," reducing the patient's psychological stress level^[23]. The narrative nursing implemented in this study required nursing staff to respect and listen to the stories behind each issue related to the patient, listening to the patient's stories with an attitude of equality and respect, while encouraging them to narrate their diagnosis and anticancer experiences, allowing patients to vent negative emotions, promoting patients' acceptance and adaptation to the disease^[24]. Psychological hope, as a protective psychological resource, plays a key role in cancer patients' stress responses to pressure and crisis events. The premise for patients to adapt to life after being diagnosed with cancer is to accept the disease; a high level of disease acceptance not only helps cancer patients better adapt to the disease but also contributes to improving patient treatment compliance, prognosis, and quality of life^[25]. Through patient narratives, nursing staff can fully understand the patient's situation and physical and mental state, grasp the key points of nursing, deeply explore the crux of the patient's current problems, and then adopt targeted nursing measures based on the patient's situation, externalizing the patient from the negative emotions of cancer, viewing the problem objectively, effectively improving the patient's psychological hope levels and disability acceptance.

5. Conclusion

Narrative nursing can effectively improve the body image of patients with breast absence after radical breast cancer surgery, increase patients' disability acceptance and psychological hope levels, reduce the psychological distress and stress levels of patients with breast absence after radical breast cancer surgery, improve patient treatment enthusiasm and rehabilitation outcomes, and is worthy of clinical promotion. However, this study also has certain limitations:

- (1) This study only selected patients for investigation of body image levels one month after surgery and conducted narrative nursing interventions. Breast cancer treatment is a long and tortuous process, during which the patient's negative emotions fluctuate with changes in the condition. Future studies can track patients at multiple time points for body image levels and trigger narrative nursing interventions on time and their application value;
- (2) This study only selected patients from one medical institution, and there is still a limitation in the representativeness of the population in terms of medical resource distribution and regional social differences. Future research can consider expanding the scope of sample distribution, extending the follow-up time, and accurately identifying the long-term body image symptoms of patients with breast absence after radical breast cancer surgery, providing a reference for the development of more comprehensive narrative nursing intervention measures.

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Research Progress on the Antitumor Effects of Individual Herbs in the Zhuang Medicine Formula Weiduqing

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Abstract: Gastric cancer is a prevalent malignant tumor that typically originates in the mucosal layer or near the gastric wall. If left untreated, it may spread to other organs, posing a severe threat to life. Currently, the primary treatment methods for gastric cancer are Western medical approaches, including surgical intervention, radiotherapy, chemotherapy, and biological targeted therapy. While these methods are highly targeted, they often come with significant adverse effects. Traditional compound formulas, with their multi-target and holistic regulatory advantages, can serve as primary or supplementary treatments for gastric cancer. They are also effective in mitigating side effects and are gaining increasing attention. In recent years, the research team has discovered that the Zhuang medicine formula Weiduqing demonstrates definitive efficacy in inhibiting gastric cancer tumors, with fewer toxic and side effects. This makes it a promising anti-cancer remedy with significant development potential. This article reviews the anti-tumor active components of individual herbs in the Weiduqing formula, providing a reference for its clinical promotion and further research.

Keywords: Zhuang medicine formula; Weiduqing; Anti-tumor ingredients; research progress

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1. Introduction

Gastric cancer is a common malignant tumor of the digestive tract, ranking among the top three in both incidence and mortality rates of malignant tumors in China. Over the past 30 years, the incidence and mortality rates of gastric cancer in the country have been on the rise, posing a severe threat to human health ^[1]. While modern medical treatments for gastric cancer are highly targeted, they often come with significant adverse effects. Therefore, finding effective treatments with minimal side effects has become a focus of research. With advances in molecular biology and traditional medicine extraction technologies, Chinese medicine and ethnic medical formulas have garnered attention for their efficacy and low toxicity. Weiduqing, a Zhuang medicine formula developed by Professors Yuzhou Pang and Yingcai Wei through over a decade of clinical

practice, has shown promise in treating various chronic gastric diseases. This formula comprises ten medicinal ingredients, including Jiubiyang, *Curcuma zedoaria*, *Solanum lyratum*, *Scutellaria barbata*, *Panax notoginseng*, *Oldenlandia diffusa*, *Coptis chinensis*, with small amounts of *Corydalis yanhusuo*, *Pseudostellaria heterophylla*, and *Glycyrrhiza uralensis*. The formula integrates detoxification and immune enhancement, promoting gastrointestinal function recovery and exhibiting anticancer effects ^[2]. Experimental studies have confirmed that Weiduqing significantly inhibits tumor growth in nude mice with transplanted gastric cancer, with its mechanism potentially related to the expression levels of the key factors PTGS2, IL6, and ICAM1 mRNA ^[3]. Based on this, the present review organizes relevant literature to summarize the antitumor active ingredients in each component of Weiduqing, providing a reference for further research on this formula.

2. Research on the main antitumor components of individual herbs in Weiduqing

2.1. *Ilex rotunda*

Ilex rotunda Thunb., Zhuang name Maexndeiheij, is included in the “Quality Standards for Zhuang Medicines in Guangxi Zhuang Autonomous Region (Volume 2).” It is the dried bark or root bark of the plant *Ilex rotunda* from the Aquifoliaceae family. The herb is cold in nature and bitter in taste, with functions including clearing heat and detoxifying, reducing swelling and relieving pain, expelling wind, and dispelling dampness. Clinically, it is often used to treat conditions such as throat swelling and pain, stomach pain caused by damp heat, and rheumatic pain. The main components of *Ilex rotunda* include saponins, terpenes, aromatic compounds, and flavonoids, among which the biologically active components are pentacyclic triterpenes and their glycosides, and phenylpropanoid glycosides ^[4]. Zhao ^[5] studied the antitumor effect of *Ilex rotunda* extract in C57 mice and found that high and medium doses of *Ilex rotunda* could significantly inhibit tumor growth, indicating that *Ilex rotunda* has a significant tumor inhibition rate. Xu ^[6] found that the triterpenoid compounds of *Ilex rotunda* have inhibitory activities on various human tumor cells such as the human nasopharyngeal carcinoma cell line, human cervical carcinoma cell line, human colon carcinoma cell line, human liver cancer cell line, human lung cancer cell line, and human breast cancer cell line *in vitro*. Nan *et al.* ^[7] showed through MTT experiments that *Ilex rotunda* acid derivatives have significant inhibitory activities on the proliferation of human malignant melanoma cells, human cervical cancer cells, human lung adenocarcinoma cells, and human liver cancer cells. According to current research, the antitumor effect of *Ilex rotunda* is mainly manifested in the treatment of liver cancer and lung cancer, while research on its anti-gastric cancer effect is relatively limited, and the specific mechanism of action still needs further investigation.

2.2. *Coptis chinensis*

Coptis chinensis, derived from the dried rhizomes of plants in the Ranunculaceae family (*Coptis chinensis*, *Coptis deltoidea*, or *Coptis teeta*), is cold in nature and bitter in taste. It is a common herb for clearing heat and drying dampness, purging fire, and detoxifying. It is often used clinically to treat damp-heat syndromes and carbuncles and abscesses. With the development of modern pharmacological experimental research methods and technologies, a substance called berberine, also known as berberine hydrochloride, has been extracted from *Coptis chinensis*, and its antitumor effect has received widespread attention and research. The experimental results conducted by Ren ^[8] showed that silencing the *IL6* gene in MKN-45 cells could significantly inhibit the proliferation of gastric cancer cells, induce cell apoptosis, and cause G0/G1 phase arrest, confirming that

berberine can inhibit the growth of gastric cancer cells through the IL-6/JAK2/STAT3 signaling pathway. Tian *et al.*^[9] stated that berberine hydrochloride can induce autophagy in gastric cancer SGC-7901 cells through the P38 MAPK signaling pathway, promoting the death of gastric cancer cells. Wang *et al.*^[10] elaborated that berberine can also inhibit various tumor cells such as colon cancer, esophageal cancer, liver cancer, bladder cancer, and brain tumors, indicating that berberine has significant anti-tumor activity against multiple tumor types.

2.3. *Solanum lyratum*

Solanum lyratum, also known as Baimaoteng or Zhuang name Gaeubwnhgauh, is included in the “Quality Standards for Zhuang Medicines in Guangxi Zhuang Autonomous Region.” This medicinal herb belongs to the Solanaceae family and is a perennial vine. The medicinal properties of this herb are slightly cold and bitter, and it has the effects of clearing heat and detoxifying, dispelling wind and removing dampness, and fighting cancer. It is often used clinically to treat breast abscesses, malignant sores, damp-heat jaundice, and ascites. Wu *et al.*^[11] observed the inhibitory effect of *Solanum lyratum* water extract on human gastric cancer SGC-7901 cells and found that with increasing doses of *Solanum lyratum*, the number of cells gradually decreased while the number of apoptotic cells gradually increased, indicating that *Solanum lyratum* water extract induces apoptosis of human gastric cancer SGC-7901 cells in a concentration-dependent manner. Liu *et al.*^[12] found through experiments that *Solanum lyratum* steroidal saponins have varying degrees of inhibitory effects on human cervical cancer HeLa cells, human ovarian cancer HO-8910 cells, human leukemia K562 cells, mouse primary ascites sarcoma S180 cells, and mouse liver cancer H22 cells *in vitro*, providing a basis and direction for further exploration of *Solanum lyratum* in the treatment of multiple cancer types. Clinically, *Solanum lyratum* is often used as a single agent or in combination with other prescriptions to treat gastric cancer and other cancers. Professor Jiuyi Xi, a famous doctor of traditional Chinese medicine in Shanghai, summarized that the Baihe Formula with *Solanum lyratum* as the main ingredient is a clinically proven prescription for the treatment of advanced gastric cancer, which has significant efficacy in treating gastric cancer, can significantly prolong the survival time of patients, and improve their clinical symptoms^[13]. Additionally, Professors Yuanfu Qi and Cunren Yu also frequently use *Solanum lyratum* compounds to treat gastrointestinal tumors^[14,15], which affirms the anti-gastric cancer effect of *Solanum lyratum* to some extent.

2.4. *Hedyotis diffusa*

Hedyotis diffusa (*Scleromitrion diffusum*), Zhuang name Nyarinngoux, is included in the “Quality Standards for Zhuang Medicines in Guangxi Zhuang Autonomous Region (Volume 1).” It belongs to the Rubiaceae family and refers to the entire plant of *Hedyotis diffusa*. The medicinal properties of this herb are cold and slightly bitter, and it has the effects of clearing heat and detoxifying, promoting urination, dispersing carbuncles and resolving masses, and diuresis. It is clinically used in the treatment of various tumors, especially gastrointestinal and lymphatic tumors^[16]. Liu *et al.*^[17] identified 12 key targets such as CA isoenzymes, P53, CDK2, PIK3CA, BCL2, AKT1, MAPK1, and VEGFA for the treatment of gastric cancer using *Hedyotis diffusa*, and pointed out that its pharmacological mechanism may be closely related to multiple biological pathways such as the VEGF signaling pathway and the PI3K/Akt/mTOR signaling pathway. Other data indicate^[18] that gastric cancer cells BGC-823 treated with total flavonoids from *Hedyotis diffusa* show typical cell apoptosis, suggesting that total flavonoids from *Hedyotis diffusa* have a strong inhibitory effect on gastric cancer cells. Another active component

of *Hedyotis diffusa*, polysaccharides, can promote cancer cell apoptosis by affecting the expression levels of *BCL2* mRNA and *P53* mRNA in human gastric cancer 7901 cells ^[19]. Additionally, *Hedyotis diffusa* polysaccharides have a significant effect on promoting apoptosis in nasopharyngeal carcinoma CNE2 cells ^[20], non-small cell lung cancer A549 cells ^[21], human laryngeal carcinoma Hep-2 cells ^[22], and skin squamous carcinoma A431 cells ^[23], indicating that *Hedyotis diffusa* also has strong antitumor activity.

2.5. *Scutellaria barbata*

Scutellaria barbata, Zhuang name Nomjsoemzsaeh, is listed in the “Quality Standards for Zhuang Medicines in Guangxi Zhuang Autonomous Region (Volume 2).” It belongs to the Lamiaceae family and refers to the entire plant of *Scutellaria barbata*. The medicinal properties of this herb are cold, pungent, and bitter, and it has the effects of clearing heat and detoxifying, dispersing blood stasis, and promoting blood circulation. It is often used in clinical practice in combination with *Hedyotis diffusa* as a common treatment for tumors ^[24]. Modern research has shown that flavonoids, polysaccharides, and diterpenes in *Scutellaria barbata* have good antitumor activity and have significant inhibitory effects on malignancies such as lung cancer, liver cancer, gastric cancer, colorectal cancer, breast cancer, sarcoma, and leukemia ^[25]. Chen *et al.* ^[26] observed that *Scutellaria barbata* polysaccharides can exert their anticancer effects by increasing P21 and reducing VEGF levels in the peripheral blood serum of gastric cancer mice, indicating that *Scutellaria barbata* polysaccharides have the ability to inhibit cancer cell proliferation and diffusion in gastric cancer mouse models. Zhang *et al.* ^[27] demonstrated that *Scutellaria barbata* extract has anti-gastric cancer SGC-7901 cell proliferation and pro-apoptotic effects, and can reduce uPA expression, slowing cancer progression and metastasis. Zhang *et al.* ^[28] found that total flavonoids from *Scutellaria barbata* can significantly inhibit gastric cancer cell proliferation and induce cancer cell apoptosis, and improve radiosensitivity. The mechanism of action may be related to the upregulation of MIIP expression. These studies have laid a theoretical foundation for the anti-gastric cancer effects of *Scutellaria barbata* and provided references for better developing its anticancer applications.

2.6. *Curcuma phaeocaulis*

Curcuma phaeocaulis, Zhuang name Guighunh, is included in the “Quality Standards for Zhuang Medicines in Guangxi Zhuang Autonomous Region (Volume 1).” It belongs to the Zingiberaceae family and refers to the dried rhizome of *Curcuma phaeocaulis*, which is one of the commonly used Zhuang medicines in clinical practice. Its medicinal properties are warm and pungent, and it has the effects of promoting qi circulation and breaking blood stasis, eliminating food stagnation, and relieving pain. It is often used for symptoms such as blood stasis-induced amenorrhea and food stagnation-induced abdominal distension. The active ingredients in the volatile oil of *Curcuma phaeocaulis*, such as curcumin and β -elemene, can affect multiple aspects of tumor cell growth, proliferation, apoptosis, and metastasis by inhibiting and regulating Ki-67, PCNA, mTOR, and miRNA, thereby slowing the progression of the tumor ^[29]. Some studies ^[30] have pointed out that the active ingredient curcumol extracted from the volatile oil of *Curcuma phaeocaulis* can significantly inhibit the proliferation of human gastric cancer BGC-823 cells and promote cancer cell apoptosis. Zhang *et al.* ^[31] confirmed that curcumol can upregulate the expression of AIF and EndoG, inducing apoptosis of gastric cancer SGC-7901 cells through a non-caspase-dependent pathway. Other studies ^[32] have found that gastric cancer AGS cells treated with curcumol have a good proliferation inhibition rate and apoptosis rate, and can simultaneously induce G0/G1 and G2/M phase arrest in gastric cancer AGS cells, slowing down their migration

and repair abilities. The realization of its effect may be related to the regulation of PI3K, p-Akt, and p-mTOR proteins, as well as the relative expression of caspase-3 and Bax, through the PI3K/Akt/mTOR signaling pathway. Wang *et al.* [33] observed that gastric cancer patients who received curcuma oil injection during the perioperative period had slower growth rates of gastric cancer cells and less severe inflammatory reactions caused by surgery. Additionally, relevant literature and research have shown that curcumol also has significant inhibitory and pro-apoptotic effects on colon cancer [34], nasopharyngeal carcinoma CNE-2 cells [35], ovarian cancer [36], and leukemia L1210 cells [37].

2.7. *Corydalis yanhusuo*

Corydalis yanhusuo is a tuberous root belonging to the genus *Corydalis* in the family Fumariaceae. This medicine has warm properties, a pungent and bitter taste, and has the effects of promoting blood circulation, regulating qi, and relieving pain. Clinically, *Corydalis yanhusuo* is mainly used to alleviate various types of pain, including cancer pain in patients with advanced tumors, cardiovascular diseases, and menstrual pain [38]. Modern research has shown that components such as corydaline, tetrahydropalmatine, berberine, coptisine, d-glucine, and d-isoboldine in *Corydalis yanhusuo* alkaloids have certain antitumor effects. Zhang *et al.* [39] reported that the total alkaloids of *Corydalis yanhusuo* significantly inhibit the proliferation of six human gastric cancer cell lines, especially AGS and MKN-28 cells. Wan [40] found that *Corydalis yanhusuo* alkaloids have strong inhibitory effects on the proliferation of four tumor cell lines: HepG2 liver cancer cells, A549 non-small cell lung cancer cell line, LOVO human colon cancer cells, and BGC-823 human gastric adenocarcinoma cells. It is believed that *Corydalis yanhusuo* alkaloids may affect VEGF expression in A549 cells through the Akt pathway. Additionally, Sang *et al.* [41] confirmed that various components of *Corydalis yanhusuo* alkaloids inhibit the proliferation of liver cancer cells SMMC-7721. Data analysis suggests that *Corydalis yanhusuo* extract can inhibit the metastasis and development of breast cancer cells by regulating the mitogen-activated protein kinase signaling pathway, demonstrating good anticancer activity [42].

2.8. *Panax notoginseng*

Panax notoginseng, Zhuang name Godienzcaet, is included in the Quality Standards for Zhuang Medicines in Guangxi Zhuang Autonomous Region (Volume 1).” It is the dried root and rhizome of the plant *Panax notoginseng* belonging to the family Araliaceae. This medicine has warm properties, a sweet and slightly bitter taste, and has the effects of regulating fire and dragon channels, stopping bleeding, nourishing blood, dispelling blood stasis, and relieving pain. It is commonly used in clinical practice to treat hematemesis, hemochezia, metrorrhagia and metrostaxis, external bleeding, and trauma-induced swelling and pain. Besides these effects, the antitumor effect of *Panax notoginseng* has also attracted the attention of many experts and scholars. Shi *et al.* [43] found through experiments that the active ingredient of *Panax notoginseng*, total saponins of *Panax notoginseng*, can inhibit the proliferation, invasion, and migration of gastric cancer cell lines through the WNT/ β -catenin pathway and induce apoptosis in gastric cancer cell lines. Cai *et al.* [44] discovered that total saponins of *Panax notoginseng* can delay the malignant progression of gastric mucosal tissue and reduce damage to the gastric mucosa of rats with precancerous lesions by inducing activation of the JNK/ERK signaling pathway. Gao *et al.* [45] found that total saponins of *Panax notoginseng* can also inhibit the proliferation of SGC-7901 cells, induce cell cycle arrest and apoptosis. Wu *et al.* [46] elucidated that total saponins of *Panax notoginseng* can inhibit proliferation and induce apoptosis in human gastric cancer MKN-28 cells *in vitro*, and initially

inferred that the induction of gastric cancer cell apoptosis by total saponins of *Panax notoginseng* is related to its upregulation of death receptor 5 expression activity. Additionally, Dr. Lijuan Liang from Jilin Provincial Hospital of Traditional Chinese Medicine used *Panax notoginseng* combined with various traditional Chinese medicinal ingredients to create medicinal diets such as Huaiqi Sanshen Decoction to prevent and treat tumors, further clarifying the antitumor effect of *Panax notoginseng*^[47]. These are all relevant elaborations on the anti-gastric cancer effects of *Panax notoginseng*, indicating that it has great potential in the treatment of gastric cancer. Furthermore, total saponins of *Panax notoginseng* have significant inhibitory effects on breast cancer tumor models in mice^[48], pancreatic cancer MIA PaCa-2 and PANC-1 cells^[49], and lung cancer cells in mice^[50].

2.9. *Pseudostellaria heterophylla*

Pseudostellaria heterophylla is the dried tuberous root of the plant *Pseudostellaria heterophylla* belonging to the family Caryophyllaceae. It has neutral properties, a sweet and slightly bitter taste, and has the effects of nourishing qi and blood, invigorating the spleen, benefiting the lungs, and relieving cough. It is commonly used in clinical practice to treat symptoms such as qi and blood deficiency, lung deficiency cough, and spleen and stomach weakness, including qi and blood deficiency syndromes caused by various types of tumors in the middle and late stages. Wang *et al.*^[51] analyzed prescriptions used by Kequn Chai in the treatment of gastric cancer and identified that *Pseudostellaria heterophylla* was one of the high-frequency herbs in these prescriptions. Common herb combinations with *Pseudostellaria heterophylla* included “*Pseudostellaria heterophylla* and licorice (*Glycyrrhiza uralensis*),” “*Pseudostellaria heterophylla* and *Atractylodes macrocephala*,” and “*Pseudostellaria heterophylla* and *Pinellia ternata*.” Additionally, the chemical constituent pseudostellarin B from *Pseudostellaria heterophylla* has been confirmed to have a certain anticancer effect. Its mechanism of action is to inhibit the PI3K/AKT signaling pathway and affect PD-L1 expression by binding to the key target CXCR4, thereby controlling the development of gastric cancer and prolonging the survival period of tumor patients^[52]. Xue *et al.*^[53] found through research that pseudostellarin B can significantly inhibit gastric cancer cell proliferation and tumor growth by activating endoplasmic reticulum stress, increasing the expression of IRE1, CHOP, and GRP78, and inhibiting the expression of Bcl-2. This suggests that pseudostellarin B may be a potential therapeutic drug for gastric cancer. Therefore, the main component of *Pseudostellaria heterophylla* with anti-gastric cancer activity is pseudostellarin B. Other studies^[54] have shown that pseudostellarin B can significantly inhibit the adhesion and invasion abilities of ECA-109 human esophageal cancer cells. Furthermore, the polysaccharide H-1-2 extracted from *Pseudostellaria heterophylla* can also inhibit the invasion and migration of pancreatic cancer cells by inhibiting hypoxia-induced AGR2 expression, which can control the progression of pancreatic cancer to some extent^[55].

2.10. *Glycyrrhiza uralensis*

Glycyrrhiza uralensis is the dried root and rhizome of the plants *Glycyrrhiza uralensis*, *Glycyrrhiza inflata*, or *Glycyrrhiza glabra* belonging to the family Fabaceae. This medicine has neutral properties and a sweet taste, and is a relatively common traditional Chinese medicine in clinical practice. It can harmonize various medicines, relieve cough and dispel phlegm, alleviate pain, clear heat, and detoxify. Modern pharmacological research has shown that the effective antitumor components of *Glycyrrhiza uralensis* mainly come from triterpenoid compounds such as glycyrrhizic acid, glycyrrhetic acid, and glycyrrhizin, as well as flavonoid compounds such as liquiritin, isoliquiritin, and glabridin^[56]. Glycyrrhetic acid can induce apoptosis in a dose-

dependent manner in gastric cancer HGC-27 cells^[57] and inhibit the growth of human gastric cancer SGC-7901 cells^[58], indicating that glycyrrhetic acid has a certain anti-gastric cancer effect. Niu *et al.*^[59] found through research that isoliquiritin can down-regulate the expression of proteins in the PI3K/AKT signaling pathway and up-regulate the downstream apoptotic protein Bax to induce apoptosis in gastric cancer SGC-7901 cells. Zhang *et al.*^[60] found through experiments that high doses of licorice flavonoids have a strong inhibitory effect on the growth of transplanted gastric cancer in nude mice and can down-regulate the expression of PCNA in gastric cancer tissues, thereby controlling the malignant progression of gastric cancer. Studies^[61] have shown that glabridin can inhibit the proliferation rate of human gastric cancer MKN-45 cells, improve the efficiency of 5-fluorouracil, and propose that P16 and the potential P16/cyclin-dependent kinase 4/cyclin D1 pathway are likely to be new targets for gastric cancer treatment. Ma *et al.*^[62] observed through clinical observation that compound glycyrrhizin injection combined with chemotherapy drugs for the treatment of gastrointestinal cancer can significantly reduce toxic reactions during chemotherapy, thereby reducing the damage of chemotherapy drugs to patients' bodies. Chen *et al.*^[63] screened Chinese medicinal ingredients that bind best to estrogen receptor α and verified their anti-gastric adenocarcinoma effects. They found that liquiritin has the strongest ability to bind to estrogen receptor α and confirmed its target and good anticancer ability. This provides further experimental evidence for the treatment of gastric adenocarcinoma with liquiritin combined with estrogen receptor α . Wang *et al.*^[64] observed that not only do glycyrrhizic acid and glycyrrhetic acid have inhibitory effects on multiple cancers such as liver cancer, lung cancer, and breast cancer, but they can also be used in combination with chemotherapy drugs to achieve synergistic effects and reduce toxicity.

3. Thoughts on the anti-tumor components and effects of Weiduqing

According to literature research, the main components with anti-tumor effects in Weiduqing include triterpenoids, berberine, total flavonoids, polysaccharides, volatile oil from *Curcuma zedoaria* and its curcumol, alkaloids, and total alkaloids, as well as total saponins from *Panax notoginseng*. These components may promote cancer cell apoptosis and affect protein expression by regulating pathways such as IL-6/JAK2/STAT3, P38 MAPK, PI3K/Akt/mTOR, and WNT/ β -catenin, thereby achieving the goal of treating tumors. Clinically, it can be used to treat tumors of the digestive system, such as esophageal cancer, gastric cancer, liver cancer, pancreatic cancer, and intestinal cancer; respiratory system tumors, such as lung cancer and nasopharyngeal carcinoma; and reproductive system tumors, such as cervical cancer and ovarian cancer.

In clinical practice, traditional compound decoctions usually require a certain amount of time for decoction. During this process, the chemical components of individual herbs may react with each other, generating new substances, and the content of various components may also change^[65]. Different chemical components and contents have various therapeutic effects in clinical practice. Therefore, in the anti-tumor research of the Zhuang medicine formula Weiduqing, it is necessary not only to study the effective components of individual herbs but also to pay attention to factors such as whether the chemical components of various herbs have synergistic effects during the decoction process and whether new compounds are generated. It is necessary to use drug-containing serum as a basis, screen out the effective blood components of the formula through methods such as high-performance liquid chromatography combined with mass spectrometry, and evaluate the pharmacological activity of each component, including binding ability to specific targets and biological activity, by combining modern research methods such as *in vitro* and *in vivo* experiments and bioinformatics, to finally reveal its

mechanism of action.

4. Conclusion and outlook

In summary, scholars at home and abroad have conducted a lot of basic research on the anti-tumor effects of individual herbs in the Zhuang medicine formula Weiduqing, and the research results show that the individual herbs of this formula have certain anti-tumor effects, laying a solid foundation for subsequent research on this formula. At the same time, the current research also has certain deficiencies, which are specifically manifested as follows: (1) The current research on individual herbs focuses on single compounds or certain types of compounds, but Chinese medicine and ethnic medicine have diverse and complex components, emphasizing the overall synergistic effect. Therefore, it is necessary to proceed from the overall perspective and discover more appropriate research models for conducting research on Chinese medicine and ethnic medicine. (2) Most of the current research on individual herbs is conducted through *in vitro* experiments (cell experiments), and some are conducted through animal experiments *in vivo*. However, there are significant differences between these experiments and the actual situation and environment after the drug enters the human body. The results of *in vitro* experiments and animal experiments have certain limitations. Therefore, it is necessary to develop models that are closer to the actual situation or conduct higher-level research (human experiments). (3) The current research focuses on the efficacy of individual herbs in Weiduqing, and there are still many gaps in toxicology research. Therefore, in future research, developing more appropriate research models, improving toxicology research, and better elucidating the anti-tumor mechanism of individual herbs in the Zhuang medicine formula Weiduqing will be of great significance.

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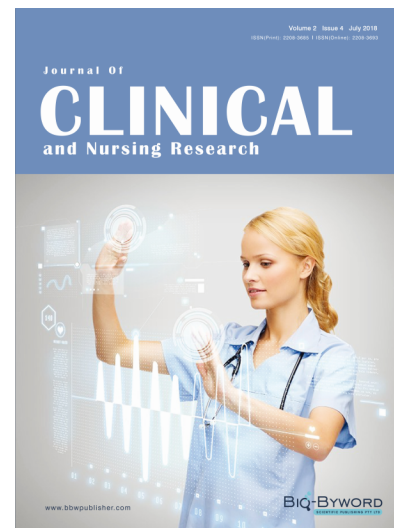
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