Article

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Analysis and Investigation of Bioinformatics and Epigenetics Reveal the Underlying Mechanisms by which FLOT2 Modulates the Progression of Diffuse Large B-cell Lymphoma

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Background: Diffuse large B-cell lymphoma (DLBCL) poses a significant threat to the quality of life for people worldwide. Regrettably, effective treatment strategies for this disease remain elusive in clinical practice due to the unclear understanding of its molecular mechanisms. Therefore, this study was devised to address these issues and identify novel diagnostic, therapeutic, and prognostic biomarkers for DLBCL.

Methods: Gene expression and clinical data for DLBCL patients were retrieved from The Cancer Genome Atlas (TCGA) database, and relevant clinical data, tumor mutational burden (TMB), and gene expression levels were extracted. Bioinformatics analysis was conducted to screen for differentially expressed genes (DEGs). The prognostic significance of flotillin-2 (FLOT2) was assessed using Kaplan-Meier survival analysis. Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot analyses were employed to evaluate mRNA and protein levels of the genes. Cell proliferation, apoptosis, and invasion were assessed using cell counting kit-8 (CCK-8) assay, flow cytometry analysis, and Transwell assay, respectively.

Results: Our bioinformatics analysis revealed that FLOT2 was significantly overexpressed in DLBCL tissues compared to normal tissues, a finding corroborated by subsequent immunohistochemistry staining, qRT-PCR, and Western blot analyses. To elucidate its biological functions, shRNAs targeting FLOT2 were transfected into DLBCL cell lines (LY-3 and U2932), resulting in suppressed cell proliferation and invasion, while promoting apoptosis. Furthermore, a positive correlation between TMB and FLOT2 expression in DLBCL was observed. Subsequently, quanTIseq was utilized to calculate the immune score and assess *FLOT2* gene expression. In DLBCL, *FLOT2* gene expression was found to be associated with T cell CD4$^+$ (non-regulatory) ($p < 0.01$), monocytes ($p < 0.05$), and uncharacterized cells ($p < 0.05$). Regarding immune checkpoint markers, including the cluster of differentiation 274 (CD274), cytotoxic T lymphocyte-associated antigen-4 (CTLA4), hepatitis A virus cellular receptor 2 (HAVCR2), lymphocyte activation gene-3 (*LAG3*), programmed cell death protein 1 (PDCD1), programmed cell death 1 ligand 2 (PDCD1LG2), Siglec-15 (SIGLEC15), and T cell immunoreceptor with Ig and ITIM domains (TIGIT), our analysis indicated that in DLBCL, FLOT2 exhibited a relationship only with TIGIT ($p < 0.05$).

Conclusions: In summary, FLOT2 functions as an oncogene and is linked to DLBCL prognosis and the tumor microenvironment. Targeting FLOT2 deletion emerges as a novel strategy to impede DLBCL aggressiveness by inhibiting cell proliferation and invasion, ultimately inducing apoptotic cell death.

Keywords: FLOT2; diffuse large B-cell lymphoma; immune checkpoint; tumor immune microenvironment; oncogene

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most prevalent type of non-Hodgkin lymphoma (NHL) among adults, constituting approximately 30% of all NHL cases [1]. This aggressive cancer originates from B cells, a type of white blood cell crucial for combating infections [2]. DLBCL can manifest in various body parts, including the lymph nodes, spleen, bone marrow, and gastrointestinal tract [3]. Common symptoms encompass enlarged lymph nodes, fever, night sweats, fatigue, weight loss, and general weakness [3]. Diagnosis of DLBCL typically involves a combination of clinical examination, imaging tests (such as CT scans and PET scans), and biopsy of affected tissue [3].

Treatment for DLBCL typically depends on the tumor node metastasis (TNM) stage of the cancer and some risk factors, including age and immunologic function [4]. The primary treatment approach involves chemotherapy, often combined with immunotherapy or targeted therapy [5]. In certain cases, radiation therapy may also be employed. For refractory or advanced disease, stem cell transplantation is a consideration [6]. The prognosis for DLBCL patients varies based on factors such as TNM stage, age, immunologic function, and others. With treatment, approximately 60–70% of DLBCL patients can achieve long-term remission, though the risk of relapse remains [7]. Ongoing clinical trials and newer therapies offer promise for improved outcomes in the future.

Flotillin-2 (FLOT2) is a protein belonging to a family of lipid raft-associated proteins involved in various cellular functions, such as signal transduction, membrane trafficking, and cytoskeletal organization [8]. In recent years, there has been growing interest in the role of FLOT2 in cancer, with studies indicating its potential involvement in tumor growth and progression [9].

FLOT2 may contribute to cancer by promoting cell proliferation and survival [10]. Research has demonstrated that FLOT2 interacts with several signaling molecules, including receptor tyrosine kinases and downstream effectors of the phosphatidyl-inositol 3-kinase/serine-threonine kinase (PI3K/Akt) pathway, which are commonly dysregulated in tumors [11]. Through these interactions, FLOT2 potentially amplifies cell signaling, thereby hastening the proliferation and survival of cancer cells.

FLOT2 may further contribute to cancer by regulating the invasiveness and metastatic potential of cancer cells [12]. Research indicates that FLOT2 expression is frequently elevated in metastatic tumors compared to primary tumors and may facilitate the formation of invadopodia, cellular structures crucial for cancer cell invasion [13].

Moreover, FLOT2 has been implicated in maintaining the stemness of cancer stem cells, which are thought to govern tumor initiation and recurrence [14]. FLOT2 likely enhances the self-renewal and survival of these cells, thereby promoting tumor growth and therapy resistance. Although FLOT2's function in blood cancers remains incompletely understood, recent research has shed light on its potential roles. One proposed mechanism is its involvement in signaling pathways. FLOT2 interacts with various signaling molecules, including protein kinases and phosphatases, which are pivotal in cell proliferation, differentiation, and survival. Through these interactions, FLOT2 may modulate their activity, contributing to the dysregulation of pathways commonly observed in cancer cells.

Additionally, FLOT2 has been implicated in regulating tumor epithelial-mesenchymal transition (EMT), a critical process for cancer cell metastasis, involving cell adhesion and migration [9]. FLOT2 likely influences these processes by interacting with other proteins, such as integrins and focal adhesion kinase.

While the precise function of FLOT2 in blood cancer, particularly in DLBCL, is still being clarified, its involvement in signaling pathways, cell adhesion, and migration implies a potentially significant role in the development and progression of DLBCL.

Materials and Methods

TCGA Data Retrieval and Data Preprocessing

The gene expression data, along with corresponding clinical information for DLBCL patients, were acquired from The Cancer Genome Atlas (TCGA) database (https://tcga-data.nci.nih.gov/tcga/). The TCGA dataset for DLBCL encompasses gene expression profiles generated through diverse high-throughput sequencing platforms, such as RNA-seq, along with associated clinical data. Utilizing suitable bioinformatics tools and pipelines, the gene expression data from TCGA were downloaded and processed [15]. Specifically, the expression levels of FLOT2 were extracted from the TCGA dataset to facilitate subsequent analyses. A total of 48 DLBCL patients had been included in the TCGA dataset, and 24 of them were characterized with high-expressed FLOT2, and the rest of them (N = 24) were featured with low-expressed FLOT2. All the experiments were conducted in accordance with the Declaration of Helsinki.

Survival Analysis

Survival analysis was performed to evaluate the association between FLOT2 expression levels and patient survival outcomes in DLBCL. Patients were stratified into low and high FLOT2 expression groups based on the median expression value. Kaplan-Meier survival curves were constructed to illustrate the survival outcomes between these two groups. Statistical significance of the survival disparities and calculation of hazard ratios was determined using log-rank tests.

Cell Culture and Vectors Transfection

This study was conducted following approval from our institutional review board. DLBCL cell lines LY-3 (#SNL-226, SUNNCELL, Wuhan, China), U2932 (#YC-C059, YuanJing Biotech, Changsha, China), WIL2S (#mCells-(C)6175, Qiming Biotech, Shanghai, China), RCK-8 (#YS3985C, Yaji Biotech, Shanghai, China) and DHL-6 (#ORC0768, Aoruisai Biotech, Shanghai, China) were procured and authenticated by STR profiling analysis and mycoplasma detection. LY-3, WIL2S, RCK-8 and DHL-6 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Cat. 30044333, Gibco, Waltham, MA, USA) and 1% penicillin-streptomycin (PS, ThermoFisher Scientific, Waltham, MA, USA), while U2932 cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 20% FBS and 1% PS. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2. Additionally, shRNA targeting FLOT2 (5′-GAG GUU GUG CAG CGC AAU U-3′) and shRNA-NC were designed and synthesized by Sangon Biotech (Shanghai, China). These were introduced into DLBCL cells using Lipofectamine 2000 transfection reagent (Invitrogen, San Diego, CA, USA) following the manufacturer's protocol.

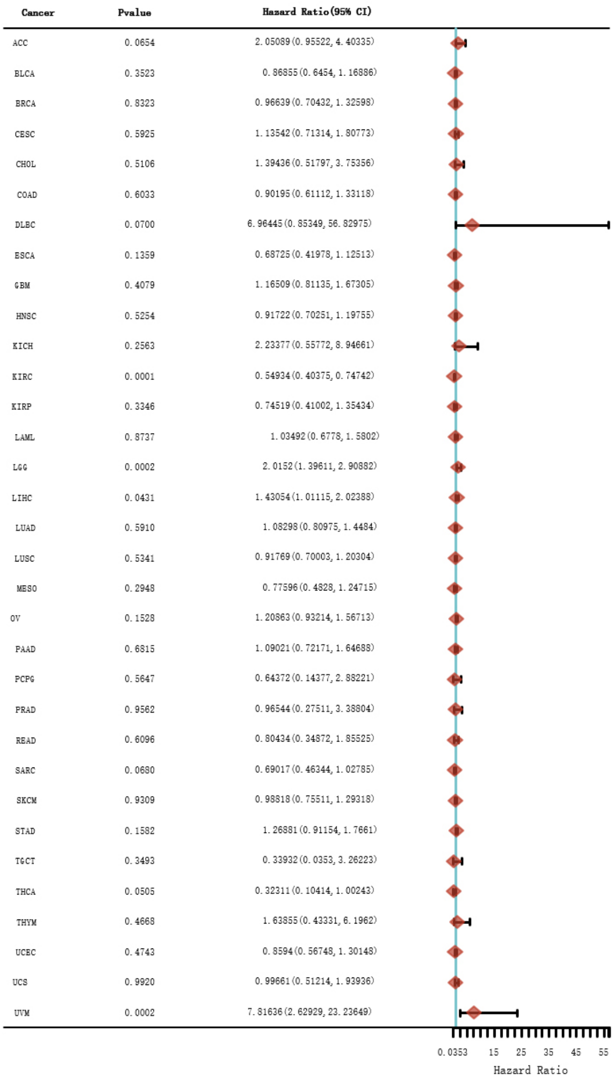

Fig. 1. The Forest plot. The results of p value, risk coefficient and 95% confidence interval of FLOT2 in different types of cancer in TCGA using univariate cox regression. FLOT2, flotillin-2; TCGA, The Cancer Genome Atlas; ACC, Adenoid cystic carcinoma; BLCA, Bladder cancer; BRCA, Breast cancer; CESC, Cervical squamous cell carcinoma; CHOL, Cholagio carcinoma; COAD, Colorectal cancer; DLBC, Diffuse large B-cell lymphoma; ESCA, Esophageal squamous carcinoma; GBM, Glioblastoma; HNSC, Head and neck squamous cell carcinoma; KICH, Kidney chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney papillary cell carcinoma; LAML, Acute myeloid leukemia; LGG, Low-grade glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesonephric-like adenocarcinomas; OV, Ovarian cancer; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma of adrenal; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin cutaneous melanoma; STAD, Stomach adenocarcinoma; TGCT, Giant cell tumor oftendon sheath; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine corpus endothelial carcinoma; UCS, Uterine carcinosarcoma; UVM, Ocular melanomas.

Gene Expression Analysis

Total RNA was extracted from DLBCL cells (LY-3 and U2932) and clinical tissues using a commercially available RNA extraction kit (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The quantity and quality of the extracted RNA were assessed using NanoDrop. Complementary DNA (cDNA) synthesis was carried out using a reverse transcription kit with a random hexamer primer. Quantitative real-time polymerase chain reaction (qRT-PCR) was then conducted to assess the gene expression levels of *FLOT2*. Specific primer pairs for *FLOT2* (Forward: 5′-CCC CAG ATT GCT GCC AAA-3′; Reverse: 5′-TCC ACT GAG GAC CAC AAT CTCA-3′) and β-actin (Forward: 5′-CTC CAT CCT GGC CTC GCT GT-3′; Reverse: 5′-GCT GTC ACC TTC ACC GTT CC-3′) were utilized. β-actin served as the endogenous reference gene, and the relative expression levels of *FLOT2* were determined using the comparative $2^{-\Delta\Delta\mathrm{Ct}}$ method.

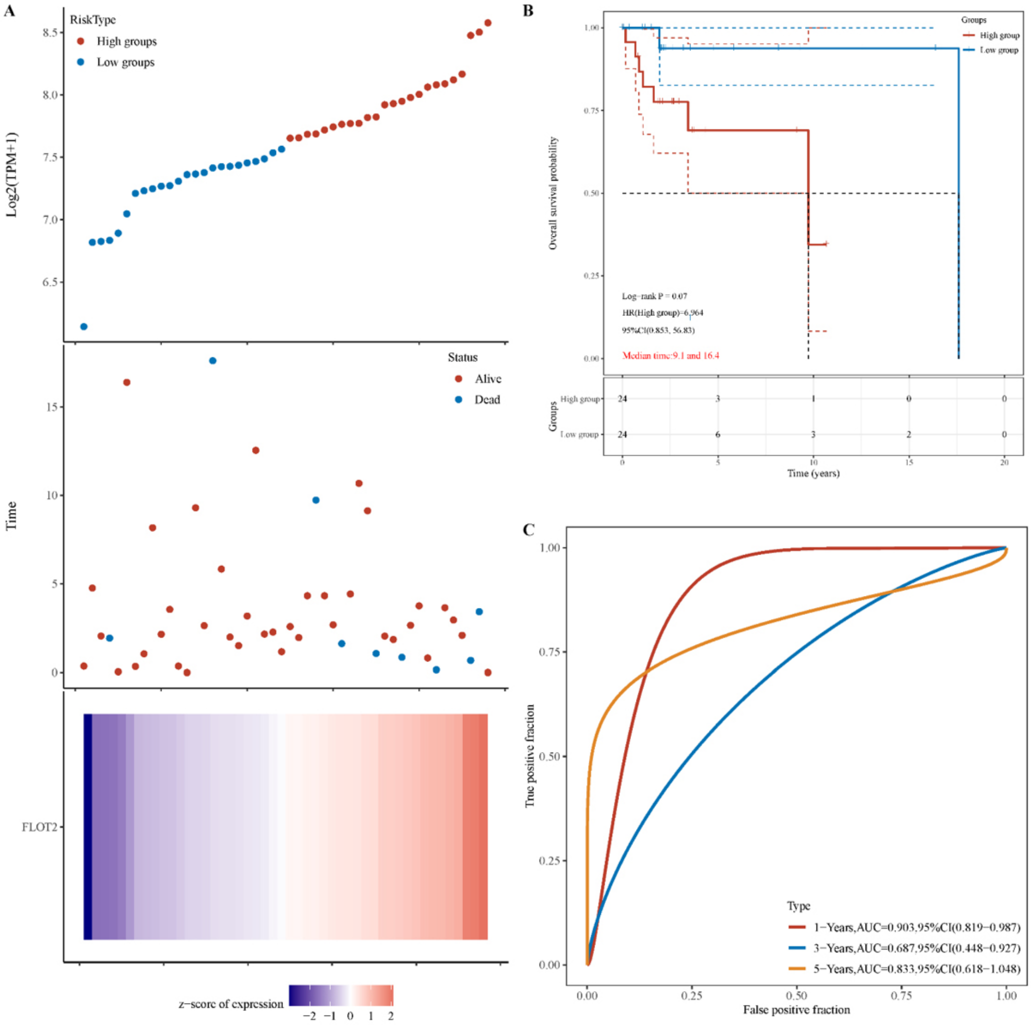

Fig. 2. The prognostic value of FLOT2 in DLBCL. (A) The DLBCL patients were divided into high group and low group according to the relative expression level of FLOT2. (B) The Kaplan-Meier survival of the above two groups. (C) The 1-, 3-, and 5-year AUC curves. DLBCL, diffuse large B-cell lymphoma.

Protein Expression Analysis

Protein extraction was carried out using a commercially available protein extraction kit as per the manufacturer's instructions. The protein concentration was determined using the Bradford assay. Subsequently, protein samples underwent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for separation. Following electrophoresis, proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane using a wet transfer system. The PVDF membrane was blocked with 5% non-fat milk and then probed with primary antibodies against FLOT2 (1:1500, #ab96507, Abcam, Cambridge, UK) and β-actin (1:2000, #ab8227, Abcam, Cambridge, UK). After primary antibody incubation, the membrane was washed and incubated with a secondary antibody conjugated to horseradish peroxidase (1:2500, #ab288151, Abcam, Cambridge, UK). Protein bands were visualized using an ECL substrate (YEASEN, Shanghai, China), and the signals were captured. Quantification of protein expression levels was performed using densitometry analysis with Image J software (Version 1.8.0, National Institutes of Health, Bethesda, MD, USA).

Cell Counting Kit-8 (CCK-8) Assay

The CCK-8 proliferation detection kit (#C0037, Beyotime, Shanghai, China) was utilized to assess the proliferative capacity of DLBCL cells. Briefly, DLBCL cells were seeded in 96-well plates at a density of 5000 cells/well and cultured for 5 days. Following incubation, the cells were treated with the CCK-8 reaction solution. Subsequently, a microplate reader (ThermoFisher Scientific, Waltham, MA, USA) was utilized to measure the optical density values at a wavelength of 450 nm, in accordance with the manufacturer's instructions. These values represented the relative proliferation abilities of the DLBCL cells.

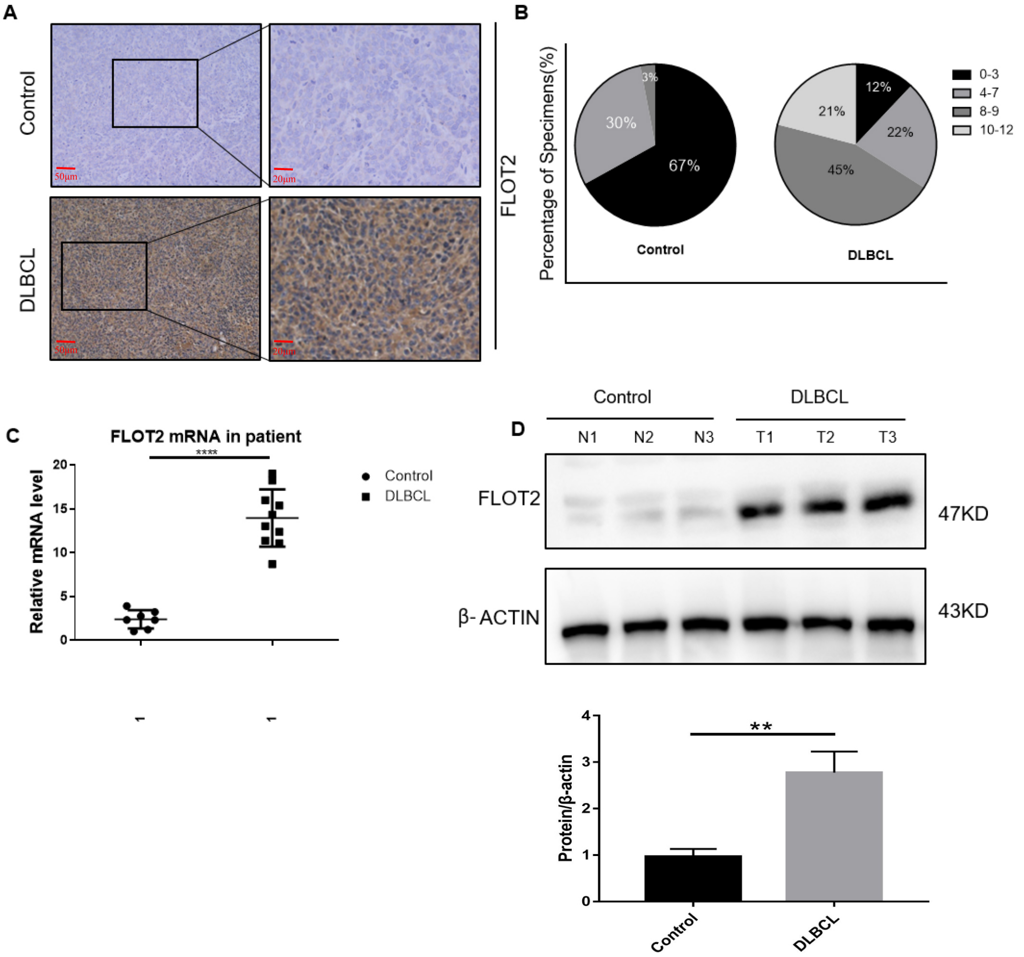

Fig. 3. FLOT2 is high expressed in DLBCL tumor tissue. (A) Immunohistochemical staining for FLOT2 expression was higher in DLBCL tissue (n = 10) than that in control samples (n = 7). Scale bars = 20 µm. (B) The distributions of immunohistochemical score. (C) The mRNA expression level of FLOT2 in DLBCL samples (n = 10) is higher than that in control samples (n = 7) ($p < 0.0001$). (D) The protein expression level of FLOT2 in DLBCL samples (n = 3) is higher than that in control samples (n = 3). p-value < 0.05 was considered as statistical significance. Note: N, Normal tissues; T, Tumor tissues. $^{**}p < 0.01$, $^{****}p < 0.0001$.

Transwell Assay

The Transwell chambers were procured from Corning (REF 3415, Shanghai, China) for conducting the Transwell assay. DLBCL cells (4×10^4) were seeded in the upper chamber of the Transwell system in 100 µL of IMDM medium without FBS supplementation. The lower chamber was filled with 500 µL of IMDM medium containing 10% FBS. Matrigel-coated membranes with 8 µm pore size separated the upper and lower chambers. The cells were cultured in the Transwell system for 24 hours. Subsequently, the membranes were retrieved and fixed with 100% methanol for 5 minutes at room temperature, followed by staining with 0.1% crystal violet for 10 minutes at room temperature. The stained cells on the membranes were counted using a light microscope (ThermoFisher Scientific, Waltham, MA, USA) to assess the invasion abilities of the DLBCL cells.

Flow Cytometry (FCM) Analysis

The commercial Apoptosis kit (#A23202, Invitrogen, San Diego, CA, USA) was obtained to assess the apoptosis ratio of DLBCL cells following the manufacturer's protocols. In brief, DLBCL cells, both with and without FLOT2-shRNA transfection, were diluted in the binding buffer and incubated with Annexin V-FITC and propidine iodide (PI) reagent at room temperature for 30 minutes in darkness. Subsequently, a flow cytometer (BD Bioscience, San Jose, CA, USA) was utilized to examine the ratio of Annexin V-FITC/PI-positive DLBCL cells.

Immunohistochemistry (IHC) Staining Assay

DLBCL tissues (N = 10) and normal tissues (N = 7) were collected from Harbin Medical University Cancer Hospital. The tissues were dissected, fixed in 10% formalin, and sliced into sections with a thickness of 4 µm, followed by antigen retrieval. Subsequently, the tissues were

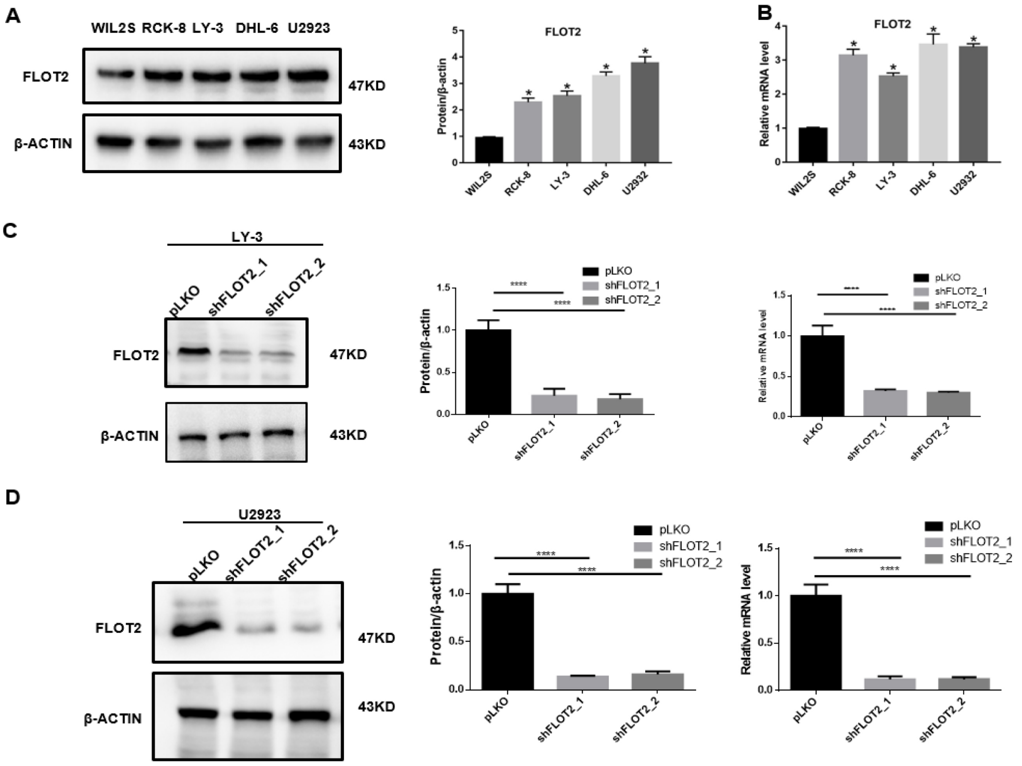

Fig. 4. The expression levels of FLOT2 in DLBCL cells. (A) The protein expression level of FLOT2 in DLBCL cell lines (n = 3). (B) The mRNA expression level of FLOT2 in DLBCL cell lines (n = 3). (C) The FLOT2 knockdown efficacy in LY-3 were confirmed by western blot and quantitative real-time polymerase chain reaction (qRT-PCR) analyses (n = 3). (D) The FLOT2 knockdown efficacy in U2923 were confirmed by western blot and qRT-PCR analyses (n = 3). *p*-value $<$ 0.05 was considered as statistical significance. **p* $<$ 0.05, *****p* $<$ 0.0001.

incubated sequentially with a primary antibody against FLOT2 (1:3000, #ab96507, Abcam, Cambridge, UK) at 4 °C overnight and an HRP-conjugated secondary antibody (1:3000, Dako, Copenhagen, Denmark) for 2 hours at room temperature. A light microscope (ThermoFisher Scientific, Waltham, MA, USA) was used to examine the ratio of FLOT2-positive cells in the tissues. All clinical experiments were conducted with approval from the Ethics Committee of Harbin Medical University Cancer Hospital (Approval no. 20210038-HTH-2213), and informed consent was obtained from all participants.

Statistical Analysis

The data were collected and presented as Means ± Standard Deviation (SD). Statistical analyses were performed using SPSS 18.0 software (IBM., Chicago, IL, USA), and visualization was done using GraphPad Prism 8.0 software (GraphPad, San Diego, CA, USA). Differences in means between two groups were analyzed using Student's *t*-test, while one-way Analysis of Variance (ANOVA) analysis was conducted to compare means from multiple groups (>2). Post hoc tests were performed using Tukey's post-hoc analysis. Statistical significance was considered when $p < 0.05$.

Results

From the TCGA database, we initially investigated the hazard ratio (HR) of FLOT2 for overall survival (OS) across various cancer types (Fig. 1). Notably, in DLBCL, FLOT2 exhibited a critical *p*-value (p = 0.07, HR = 6.96445, 95% CI 0.85349–56.82975). Subsequently, we divided patients into high and low expression groups based on the median value of FLOT2 expression (Fig. 2A). Kaplan-Meier survival curves revealed that patients with high FLOT2 expression had relatively poorer outcomes (p = 0.07, HR = 6.964, 95% CI 0.853–56.83) (Fig. 2B). Furthermore, ROC curves were constructed for FLOT2 to predict OS, yielding 1-, 3-, and 5-year AUC values of 0.903 (95% CI 0.819–0.987), 0.687 (95% CI 0.448–0.927), and 0.833 (95% CI 0.618–1.048), respectively (Fig. 2C).

Immunohistochemical staining revealed higher expression of FLOT2 in DLBCL tissues compared to control samples (Fig. 3A), with the distribution of Immunohistochemical scores depicted in Fig. 3B. In the control group, the scores ranged from 0–3 (67%), 4–7 (30%), to 8–9 (3%), while in DLBCL, scores ranged from 8–9 (45%), 4–7 (22%), 10–12 (21%), to 0–3 (12%) (Fig. 3B). Moreover, FLOT2 expression was explored in patient samples. The mRNA expression level of FLOT2 in DLBCL samples (n =

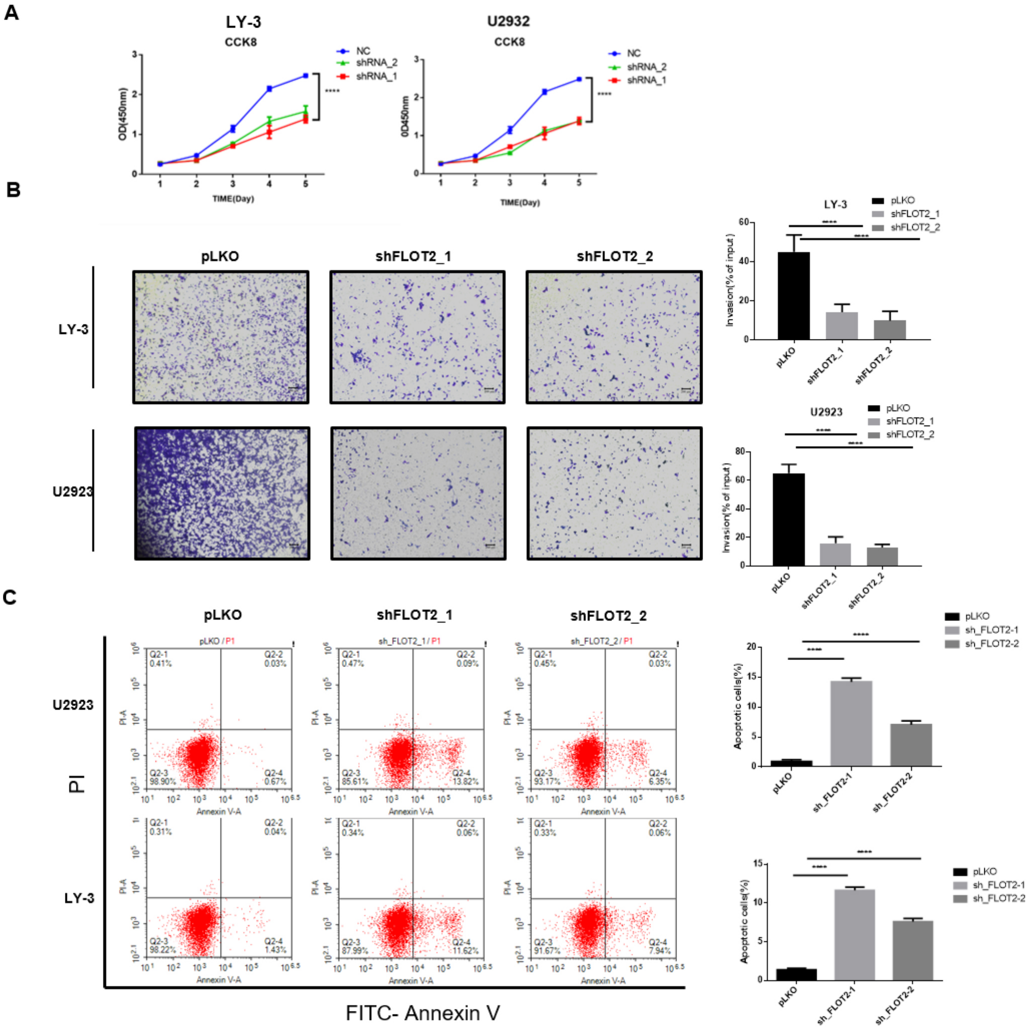

Fig. 5. FLOT2 acts as an oncogene in DLBCL. (A) The cell proliferation viability was monitored in LY-3 and U2923 cell lines (n = 3). (B) The migration viability (n = 3), and the length of the scale bars are 200 µm. (C) The cell apoptosis was also explored (n = 3). *p*-value < 0.05 was considered as statistical significance. *****p* < 0.0001.

10) was significantly higher than that in control samples (n = 7) ($p < 0.0001$, Fig. 3C). Similarly, the protein expression level of FLOT2 in DLBCL samples was elevated compared to control samples (Fig. 3D).

To delve deeper into the functional role of FLOT2 in DLBCL, we assessed its expression levels in various DLBCL cell lines, including WIL2S, RCK-8, LY-3, DHL-6, and U2923 (Fig. 4A, $p < 0.05$). Additionally, we explored the relative mRNA levels of FLOT2 in these cell lines (Fig. 4B, $p < 0.05$). Subsequently, we established stable FLOT2 shRNA-expressing cell lines (LY-3 and U2923) through lentiviral infection. The efficacy of two shRNA oligonucleotides was validated via western blot and qRT-PCR analyses (Fig. 4C,D, $p < 0.0001$).

In the CCK-8 assay, we observed that FLOT2 knockdown significantly inhibited cell proliferation ability in both LY-3 and U2923 cell lines (Fig. 5A, $p < 0.0001$). Similarly, FLOT2 knockdown resulted in reduced cell migration ability in LY-3 and U2923 cell lines, as demonstrated by the Transwell assay (Fig. 5B, $p < 0.0001$). Moreover, FLOT2 knockdown promoted cell apoptosis in LY-3 and U2923 cell lines (Fig. 5C, $p < 0.0001$).

To probe into the potential mechanism of FLOT2 in DLBCL, we first conducted Spearman correlation analysis of tumor mutational burden (TMB) and FLOT2 expression. Our analysis revealed a positive correlation between TMB and FLOT2 expression in DLBCL (Fig. 6A). Next, we utilized quanTIseq to calculate the immune score and *FLOT2* gene expression. In DLBCL, we found that *FLOT2* gene expression is associated with T cell CD4$^+$ (non-regulatory) ($p < 0.01$), monocyte ($p < 0.05$), and uncharacterized cell ($p < 0.05$) populations (Fig. 6B).

Regarding immune checkpoint markers (cluster of differentiation 274 (CD274), cytotoxic T lymphocyte-associated antigen-4 (CTLA4), hepatitis A virus cellular receptor 2 (HAVCR2), lymphocyte activation gene-3 (*LAG3*), programmed cell death protein 1 (PDCD1), programmed cell death 1 ligand 2 (PDCD1LG2), Siglec-15 (SIGLEC15),

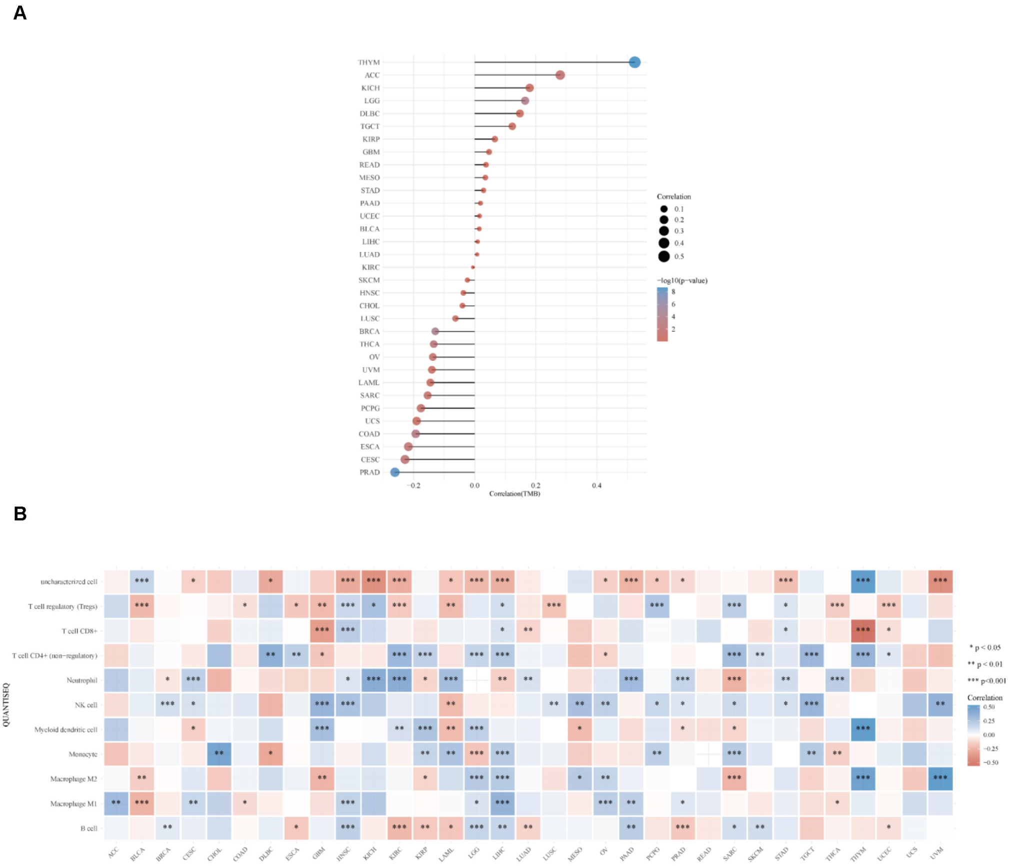

Fig. 6. FLOT2 is relevant to tumor immunity. (A) The Spearman correlation analysis of TMB and FLOT2 expression. (B) The correlation analysis between FLOT2 and immune cells among different types of cancers using quanTIseq. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. TMB, tumor mutational burden.

and T cell immunoreceptor with Ig and ITIM domains (TIGIT)), our analysis revealed that in DLBCL, FLOT2 is only correlated with TIGIT ($p < 0.05$) (Fig. 7).

Discussion

Diffuse large B-cell lymphoma (DLBCL) stands as the most prevalent form of non-Hodgkin's lymphoma in adults, categorized into germinal center B (GCB) and non-GCB subtypes, with the majority of non-GCB being activated B cell-like (ABC type) [16]. While many patients attain long-term survival with R-CHOP treatment, some display insensitivity to treatment or relapse afterward. In recent years, the tumor microenvironment has emerged as a pivotal factor influencing DLBCL occurrence and progression [17].

Lipid rafts, small regions approximately 10–200 nm in diameter on the cell membrane, are abundant in cholesterol, sphingomyelin, caveolins (CAVs), flotillin-1 (FLOT1), flotillin-2 (FLOT2), prohibitin (PHB), and other constituents. These lipid raft-associated proteins, including stomatin (EPB72)-like 2 (STOML2), partake in numerous physiological processes such as cell membrane transport and signal transduction [18]. Recent investigations have unveiled upregulation of lipid raft proteins in various tumors, suggesting their involvement in tumorigenesis and progression [19]. Among these lipid raft proteins, FLOT1 has garnered attention for its upregulation in a spectrum of malignant tumors and its regulatory roles [20,21]. However, the expression pattern of FLOT2 in DLBCL, along with its biological functions and molecular regulatory mechanisms, remains elusive.

FLOT2 has recently been implicated in the onset and progression of various malignant tumors, such as breast cancer [22], melanoma [23], cervical cancer [24], lung cancer [25], liver cancer [8], kidney cancer [26], and gastric cancer [27]. This suggests that FLOT2 may play a significant role in tumor development. In our research, we observed that patients with high expression levels of FLOT2 tended to experience poorer outcomes, as indicated by Kaplan-Meier survival curves. Additionally, the expression of FLOT2 was found to be higher in patients compared to control samples, as demonstrated by both immunohistochemically staining and western blot assays.

As a novel biomarker, FLOT2 has been reported to be closely associated with the proliferation, invasion, and metastasis of malignant tumors, thus holding promise as an indicator for assessing tumor invasion, metastatic potential, and prognosis. To validate the functional role of FLOT2,

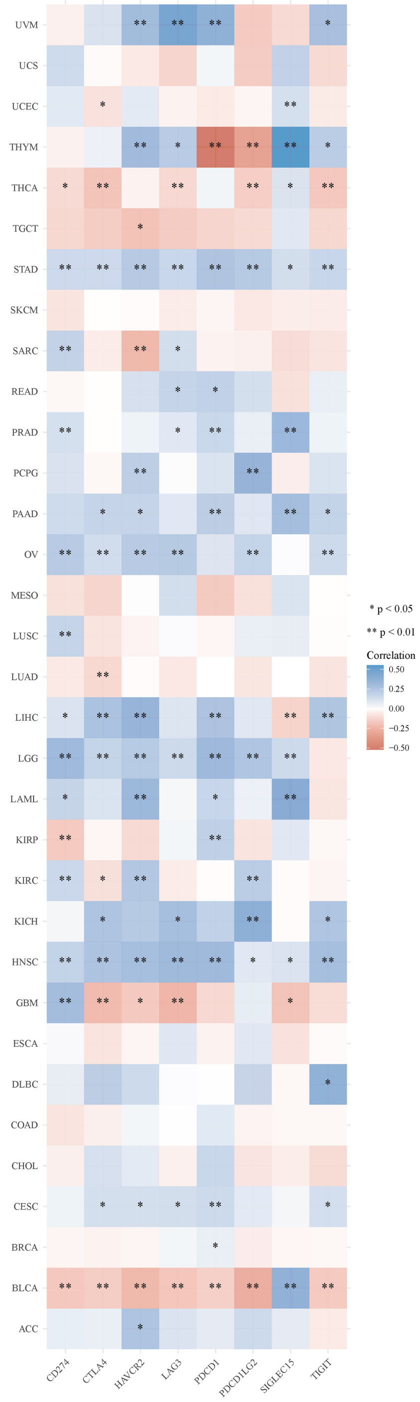

Fig. 7. FLOT2 is relevant to immune checkpoints among different tumors. In DLBCL, FLOT2 has relationship with TIGIT ($p < 0.05$). *$p < 0.05$, **$p < 0.01$. TIGIT, T cell immunoreceptor with Ig and ITIM domains.

we generated stable cell lines expressing FLOT2 shRNA. Knockdown of FLOT2 resulted in inhibited cell proliferation and migration, while promoting cell apoptosis.

Prior research has indicated that FLOT2 influences tumor invasion and metastasis through various signaling pathways, including Wnt, PI3K/Akt, NF-κB, and p53 [27,28]. It is anticipated that with further investigation into the *FLOT2* gene and a deeper understanding of the specific mechanisms underlying the invasion and metastasis of malignant tumors, *FLOT2* could serve as a target for studying gene-specific inhibitors, RNA interference carriers, and other novel therapeutic agents. This could offer promising prospects for the treatment of tumors.

In recent years, targeted therapy, cell therapy, and other approaches have offered new hope to patients with DLBCL, with the use of these drugs also advancing the individualization of immunotherapy [29]. In our study, we employed quanTIseq to calculate the immune score and assess *FLOT2* gene expression in DLBCL. Our findings indicate that *FLOT2* functions as an oncogene in DLBCL, influencing cell proliferation, migration, and apoptosis.

It has been reported that FLOT2 supports the expression of the adhesion molecule CD44, thereby impacting the progression of chronic myeloid leukemia [30]. As our understanding of the biological functions of malignant tumor biomarkers, signal transduction pathways, and epigenetic regulation deepens, along with insights into their interaction with the lymphoma cell microenvironment in lymphoma progression, several new drugs and immunotherapy strategies have been under study and development.

However, numerous questions remain unresolved, including optimal treatment schedules, dosages, and combination therapies with other drugs. The widespread adoption of new immunotherapy approaches holds the potential to revolutionize the treatment landscape for malignant lymphoma in the future.

The pathogenesis of DLBCL is a multifaceted process influences by various factors [29]. Gene mutation, epigenetic remodeling, differentiation block, evasion of immune surveillance, immune infiltration, and the constitutive activation of numerous signal transduction pathways all contribute significantly to the activation and sustenance of DLBCL tumor cells [29]. Consequently, understanding the pathogenesis of DLBCL involves not only deciphering the dysregulation of oncogene expression, but also comprehending the mechanism by which tumors evade immune cell-mediated tumor surveillance [30]. Hence, identifying a prognostic marker that correlates with immune infiltration is essential for the identification of potential therapeutic targets in DLBCL.

Immunotherapy has emerged as a crucial treatment modality for DLBCL [31]. Various immunotherapies, including CD20 monoclonal antibody therapy, bispecific antibody therapy, ADC therapy, immune checkpoint inhibitor therapy, and chimeric antigen receptor T cells (CART) therapy, have demonstrated promising efficacy and safety [31]. The advent of immunotargeted drugs have broadened the treatment landscape for relapsed/refractory DLBCL, with targeted therapy focusing on the microenvironment presenting new opportunities for treatment, particularly in T cell and macrophage-related immune mechanisms, as well as in drug development [31]. Further research is imperative to fully elucidate the mechanisms underlying immunotherapy and its potential as a therapeutic target in DLBCL.

The present study represents the first comprehensive analysis of the functions and underlying mechanisms through which FLOT2 accelerates the progression of DLBCL. We demonstrated that silencing of FLOT2 induced apoptotic cell death and suppressed cell proliferation and invasion, thereby impeding the progression of DLBCL. Importantly, our findings provide evidence supporting the potential utility of FLOT2 as an ideal biomarker for the diagnosis, treatment, and prognosis of DLBCL.

Despite these significant findings, our study has two notable limitations. Firstly, while we primarily investigated this issue through bioinformatics analysis and cellular experiments, further validation through clinical and animal experiments is necessary to corroborate our conclusions *in vivo*. Secondly, while our work focused on elucidating the biological functions of FLOT2 in regulating DLBCL progression, future studies should aim to identify the downstream signaling pathways that may be regulated by FLOT2. Addressing these shortcomings will be essential for a more comprehensive understanding of FLOT2's role in DLBCL pathogenesis and its potential clinical implications.

Conclusions

In summary, our study identifies FLOT2 as an oncogene that promotes the progression of DLBCL, suggesting that silencing FLOT2 can represent a novel therapeutic strategy to impede DLBCL development. Furthermore, our findings suggest that FLOT2 holds potential as a biomarker for the diagnosis, treatment, and prognosis of DLBCL.

Availability of Data and Materials

All the data can be provided by the corresponding author if other researchers need.

Author Contributions

YJZ designed the present study, and conducted the bioinformatics analysis and drafted the first version of the manuscript. YC and QG finished the functional experiments, collected and analyzed the data, and they also significantly modified the “Materials and Methods” and “Results” sections of the paper. YZ provided technical supports and re-organized the “Discussion” section of the paper. As the corresponding author, AL provided guidance for this work, and is also responsible for the conception, design, investigation, data analysis and validation of the paper, and also re-organized the whole structure of the manuscript, and re-written the “Abstract” and “Introduction” sections of the paper. All the authors approved the final version of the manuscript, and agreed to be accountable for all aspects of this work.

Ethics Approval and Consent to Participate

All clinical experiments were conducted with approval from the Ethics Committee of Harbin Medical University Cancer Hospital (Approval no. 20210038-HTH-2213), and informed consent was obtained from all participants. All the experiments were conducted in accordance with the Declaration of Helsinki.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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