Molecular Carcinogenesis

WILEY

Molecular Carcinogenesis

RESEARCH ARTICLE OPEN ACCESS

CtBP2 Regulates Wnt Signal Through EGR1 to Influence the Proliferation and Apoptosis of DLBCL Cells

Jianfang Dong | Lihua Li | Xuefei Zhang | Xijing Yin | Zucong Chen

Department of Hematology, The People's Hospital of Dehong, Dehong Hospital Affiliated of Kunming Medical University, Dehong, Yunnan Province, China

Correspondence: Zucong Chen (czuconghem@outlook.com)

Received: 12 October 2024 | **Revised:** 13 February 2025 | **Accepted:** 23 February 2025

Keywords: CtBP2 | DLBCL | EGR1 | Wnt/β-catenin

ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is the most prevalent form of lymphoma. The overexpression of CtBP2 in tissues may contribute to tumor occurrence and progression. The expression of EGR1 in DLBCL is elevated, suggesting its potential role as an oncogene that promotes the proliferation of DLBCL cells. Database predictions indicate that CtBP2 can bind to EGR1. The objective of the present study was to investigate whether CtBP2 can influence the proliferation and apoptosis of DLBCL cells by regulating the Wnt signaling pathway through EGR1. Western blot assay showed that CtBP2 expression was upregulated in DLBCL cells. Cell proliferation level was detected by CCK8 assay and EdU staining, and the apoptosis level and cycle distribution were analyzed through flow cytometry. Our data indicated that interference with CtBP2 and EGR1 can inhibit the proliferation and cell cycle progression of DLBCL cells while promoting apoptosis. The predictions from the HDOCK server, along with the results of Co-IP experiments, suggested that EGR1 and CtBP2 can effectively bind to each other, with EGR1 positioned downstream of CtBP2 and regulated by it. Furthermore, interference with CtBP2 could also inhibit the expression of the Wnt/β-catenin signaling pathway. Overexpression of EGR1 counteracted the effects of siRNA-CtBP2, promoting cell proliferation and cycle, inhibiting apoptosis and upregulating the expression of the Wnt/β-catenin signaling pathway. From the above experiments, we found that CtBP2 can regulate the Wnt/β-catenin signaling pathway through EGR1 to influence the proliferation and apoptosis of DLBCL cells. Therefore, EGR1 may be one of the key contributors involved in the regulation of Wnt/β-catenin signaling by CtBP2.

1 | Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most prevalent form of lymphoma, characterized by significant genetic heterogeneity. This variability contributes to marked differences in clinical presentations, treatment responses and patient outcomes [1]. The mortality rate for malignant lymphoma is as high as 15%, placing it between the 11th and 13th positions among all malignant tumors in China [2]. Currently, the primary treatment strategies for DLBCL include radiotherapy, chemotherapy, hematopoietic stem cell transplantation, targeted therapy and immunotherapy. Due to the toxic and side effects of chemotherapy and a high recurrence rate, many patients ultimately discontinue the treatment, resulting in a final survival rate of only 30%–50% [3]. Consequently, there is an urgent need to identify biomarkers for the diagnosis and treatment of DLBCL.

The expression of CtBP is abundant during the developmental process, where it plays a crucial role in axial patterning, as well as in the proliferation and differentiation of various organs (such as eyes, heart, brain, and placental vascular system) [4]. CtBP functions synergistically to inhibit complex processes in various developmental stages, suggesting that the overexpression of CtBP in adult tissues may influence both tumor initiation and progression [5]. CtBP2 enhances the malignancy of osteosarcoma cells via the JAK1/Stat3 signaling pathway [6], and the interaction between CtBP2 and ZBTB18 further promotes the malignant development of glioblastoma [7]. Additionally, CtBP2 facilitates the proliferation

of non-small-cell lung cancer and diminishes drug sensitivity through the Wnt/β-catenin pathway [8]. However, it remains unclear whether CTBP2 can participate in the malignant progression of DLBCL. The results from the GSE56315 database indicated that the expression of CtBP2 was elevated in DLBCL tissue (55 in the DLBCL group compared to 33 in the control group). Therefore, this study aims to investigate whether interference with CtBP2 can impede the malignant processes of DLBCL cells by inhibiting their proliferation and inducing apoptosis.

Currently, research indicates that CtBP2 plays a biological role by binding to target proteins. For instance, the interaction between CtBP2 and ZBTB18 promotes the malignant progression of glioblastoma [7]. Additionally, CtBP2 enhances the Wnt/β-catenin signaling pathway to participate in the development of esophageal squamous cell carcinoma through its interaction with TGIF [9]. The direct interaction between CtBP2 and DvL1 has been shown to promote the proliferation of non-small-cell lung cancer while reducing the drug sensitivity [8], indicating that CtBP2 may also facilitate the malignant processes of DLBCL through its interactions with specific target proteins. The FPCLASS database predicts that CtBP2 can bind to EGR1, which has been demonstrated to promote the proliferation of interstitial epithelial cells in pancreatic cancer [10]. Furthermore, EGR1 can promote the aggressiveness of uterine cancer and is associated with poor prognosis by inducing SOX9 expression [11]. Consequently, EGR1 is considered a potential oncogene that may drive the proliferation of DLBCL cells.

Immunohistochemical analysis revealed that the expression level of EGR1 in DLBCL was higher than that in normal lymphoid tissue, and the expression level of EGR1 in the activated B cell-like (ABC) subtype was greater than that in the germinal center B cell-like (GCB) subtype [12]. Additionally, EGR1 has been shown to activate the Wnt signaling pathway [13]. Therefore, this study aims to explore the mechanism of CtBP2 regulating Wnt signal through EGR1 and affecting the proliferation and apoptosis of DLBCL cells.

2 | Materials and Methods

2.1 | Cell Culture

GM12878, U2932, OCI-LY3, OCI-LY8, and OCI-LY10 cells were all purchased from iCell Bioscience. GM12878 and U2932 cells were cultured in RPMI 1640 (11875119, Gibco; Thermo Fisher Scientific Inc.) (supplemented with 10% FBS), while OCI-LY3, OCI-LY8, and OCI-LY10 cells were grown in IMDM (12440061, Gibco; Thermo Fisher Scientific Inc) (containing 20% human serum). The cells were maintained in a humidified incubator at 37°C with 5% CO_2. The culture medium was changed or passaged regularly based on the condition of the cells.

2.2 | Cell Transfection

The specific siRNAs, siRNA-CtBP2, and siRNA-EGR1, targeting CtBP2 and EGR1, respectively, along with the corresponding control siRNA (siRNA-NC), were synthesized by GenePharma. The silencing of CtBP2 or EGR1 in OCI-LY10 cells was achieved through siRNA transfection. EndoFecti MAX transfection reagent (EF014, iGene Biotechnology Co. Ltd.) was utilized to facilitate the transient transfection of CtBP2/EGR1 siRNAs into the cells at 37°C. Forty eight hours posttransfection, the medium was replaced with a fresh culture medium to continue cell growth.

The specific overexpressed plasmid for EGR-1 (Ov-EGR1) and the corresponding control plasmid (Ov-NC) were constructed by GenePharma. EGR-1 was overexpressed in cells through plasmid transfection. Two micrograms of the overexpression plasmid were mixed with a pre-transfection reagent (Invitrogen; Thermo Fisher Scientific Inc.), and the mixture was added to the cell culture medium. After incubating at 37°C for 48 h, the medium was replaced with fresh medium to continue cell culture.

2.3 | Cell Counting Kit-8 (CCK-8) Assay

CCK-8 kit (Beyotime Technology) was utilized to evaluate the proliferation levels of the cells. After 24 h of cell transfection, 10 μL of CCK-8 reagent was added to each well, and the samples were incubated at 37°C for 1 h. The optical density value was measured at 450 nm using a microplate reader (Bio-Rad). The average value from multiple wells was calculated, and each experiment was repeated three times.

2.4 | Proliferation Level of Cells Was Observed by EDU Staining

After adjusting the cell density, the cells were inoculated into a 24-well plate and incubated for 24 h. Subsequently, they were treated with the EdU working solution (C0071, Beyotime Institute of Biotechnology) at a final concentration of 10 μmol/L at 37°C for 2 h. Following this, DAPI staining (C1006, Beyotime Institute of Biotechnology) was performed. According to the instructions provided with the EdU-488 cell proliferation detection kit, cells in each group were observed and photographed using a fluorescent microscope.

2.5 | Flow Cytometry

2.5.1 | Detection of Cell Cycle

The cells were digested with trypsin, washed with PBS, and centrifuged three times. They were then fixed with pre-cooled 70% ethanol and stored at 4°C overnight. The following day, the cells were washed by centrifugation with PBS for three times, and the supernatant was discarded. After adding 100 μg/mL RNase, the cells were bathed in water at 37°C for 30 min, followed by staining with propidium iodide (PI) staining solution (HY-D0815, MedChemExpress) for an additional 30 min. The cell cycle distribution was analyzed using flow cytometry, and the cell content in each phase was quantified and analyzed with FlowJo software.

2.5.2 | Detection of Apoptosis

The cells were digested with trypsin and collected by centrifugation. Pre-cooled PBS was used to wash the cells and prepare single-cell suspensions. The cells were then stained with 5 μL of annexin V-APC and 5 μL of V-PE (BD Biosciences), a procedure conducted at room temperature in the dark. Finally, 10 μL of propidium iodide (HY-D0815; MedChemExpress) was added, and cellular apoptosis was analyzed immediately using flow cytometry.

2.6 | Western blot (WB)

Protein was extracted from cells using RIPA buffer (WB3100, NCM Biotech Co. Ltd.), and the protein samples from each group were quantified using a BCA kit (NCM Biotech Co. Ltd.). The protein samples were loaded onto a 10% SDS-PAGE gel (P2012, NCM Biotech Co. Ltd.) and subsequently transferred to a PVDF membrane (Millipore, USA). The membrane was sealed with 5% BSA at room temperature for 2 h and then incubated with the primary antibody at 4°C overnight. The antibodies utilized in this study included CtBP2 (10346-1-AP; Proteintech), EGR-1 (22008-1-AP; Proteintech), Bcl-2 (12789-1-AP; Proteintech), Bax (ab32503; Abcam), Caspase 3 (9961, CST), cleaved-Caspase 3 (9962, CST), DKK1 (ab307367; Abcam), β-catenin (ab32572; Abcam) and c-Myc (ab185656; Abcam). The following day, the PVDF membrane was incubated with the corresponding secondary antibody at room temperature for 1 h. After washing with PBS, the protein bands were detected using an ECL detection system and protein density was analyzed using ImageJ software.

2.7 | Molecular Docking Analysis

The three-dimensional protein structures of CtBP2 and EGR-1, in PDB format, were obtained from the Protein Data Bank (http://www1.rcsb.org/) and imported into the HDOCK molecular docking server (http://hdock.phys.hust.edu.cn) for molecular docking analysis. Once the docking results are generated, click on "Top 10 Predictions" to filter the 10 most probable docking outcomes.

2.8 | Co-Immunoprecipitation (Co-IP)

Cells were collected by centrifugation at 4°C for 5 min at 500 g, washed with precooled PBS, and then lysed on ice with 500 μL of IP lysate (87788, Thermo Fisher Scientific Inc.) containing a protease inhibitor for 30 min. After centrifugation at 4°C for 30 min at 10,000 *g*, the supernatant was collected, and a small aliquot of the supernatant (approximately 10–20 μL) was reserved for the WB experiment. IgG antibody (1 μg) or IP-indicating antibody (1 μg) was mixed with the remaining supernatant and incubated overnight at 4°C to form the antigen-antibody complex. An appropriate amount of the extracted protein was used as an input control. The IP-indicating antibodies utilized in this experiment were CtBP2 (10346-1-AP; Proteintech) and EGR-1 (22008-1-AP; Proteintech).

The protein A/G beads (20422, Thermo Fisher Scientific Inc.) were prepared, and the antigen-antibody complexes were mixed with 50 μL of protein A/G beads and incubated at 4°C for 1 to 4 h. The mixture was then centrifuged at 4°C for 5 min at 3000 g to collect the precipitate. The protein A/G beads were washed with 500 μL of lysate. Following the appropriate elution method, the target protein was obtained after eluting the antigen-antibody complex, and the target protein was detected by WB.

2.9 | Statistical Analysis

All results were analyzed using GraphPad Prism 8.0. Differences between the two groups were compared using a *t*-test, while comparisons among three or more groups were conducted using one-way analysis of variance. The results are expressed as mean ± standard deviation (SD). A *p*-value of less than 0.05 indicates that the difference is statistically significant.

3 | Results

3.1 | The Expression of CtBP2 Increased in DLBCL Cells

Firstly, we detected the expression of CtBP2 in the DLBCL cell line. The results of the WB analysis indicated that the expression of CtBP2 in the U2932, OCI-LY3, OCI-LY8 and OCI-LY10 cells was higher than that in GM12878 cells, with OCI-LY10 cells exhibiting the strongest expression. Therefore, we selected OCI-LY10 cells for the follow-up experiment (Figure 1).

3.2 | Interference With CtBP2 Inhibited the Proliferation and Cycle Acceleration of DLBCL Cells

The overexpression of CtBP2 in tissues may contribute to tumorigenesis and progression [5]. To investigate this, we constructed a small interfering RNA (siRNA) targeting CtBP2 to inhibit its expression and conducted subsequent experiments. WB assay revealed that the transfection of siRNA-CtBP2 reduced the expression of CtBP in the cells. Upon comparison, we found that the transfection efficiency of siRNA-CtBP2-1 was superior, leading us to select this siRNA for further experiments (Figure 2A). The results from the CCK-8 assay and EdU staining showed a significant decrease in cell proliferation following CtBP2 interference (Figure 2B,C). We further assessed the cell cycle distribution. Flow cytometry analysis showed that after CtBP2 interference, there was an increase in the proportion of cells in the G1 phase and a decrease in the S phase, indicating a slowdown in the cell cycle (Figure 2D). These findings suggested that interference with CtBP2 inhibited the proliferation and cycle acceleration of DLBCL cells.

3.3 | Interference With CtBP2 Promoted Apoptosis of DLBCL Cells

To detect the impact of interfering CtBP2 on the apoptosis of DLBCL cells, we assessed the apoptosis levels in cells

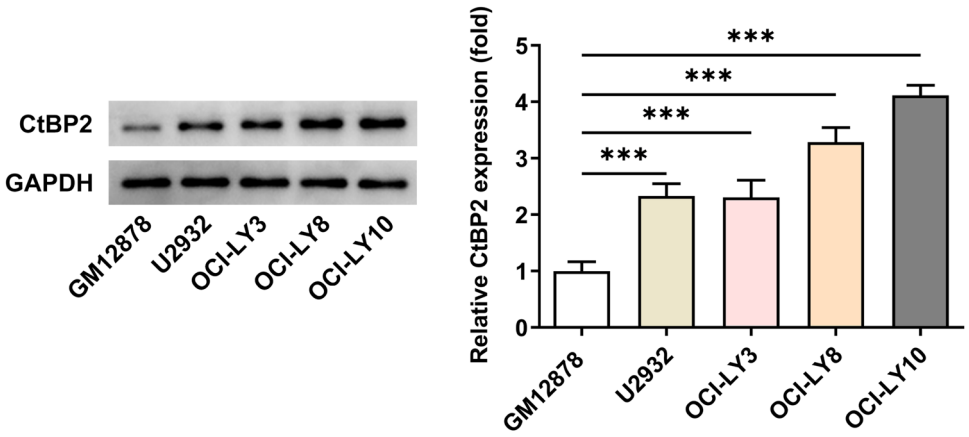

FIGURE 1 | The expression of CtBP2 increased in DLBCL cells. The expression of CtBP2 in DLBCL cell line was detected by western blot. ***$p < 0.001$. DLBCL, diffuse large B-cell lymphoma.

transfected with siRNA-CtBP2. Comparative analysis showed a significant increase in the percentage of apoptotic cells (Figure 3A). Additionally, the expression level of the anti-apoptotic protein Bcl-2 decreased, while the expression levels of proapoptotic proteins Bax and cleaved caspase-3 increased significantly (Figure 3B) after CtBP2 interference. From this, we can know that interference with CtBP2 promoted the apoptosis in DLBCL cells.

3.4 | Interference With CtBP2 Inhibited the Expression of EGR-1

Literature research shows that the expression of EGR1 is elevated in DLBCL, indicating that it may function as a potential oncogene that promotes the proliferation of DLBCL cells [12]. Additionally, database prediction also shows that CtBP2 can combine with EGR1. We utilized the HDOCK server to predict the combination between CtBP2 and EGR1, and the results revealed a combination index of 0.9 (a value greater than 0.7 indicates a very high likelihood of combination), indicating a strong potential for their combination (Figure 4A). We found that the expression of EGR-1 protein in cells significantly reduced after transfection with siRNA-CtBP2 (Figure 4B). Furthermore, the Co-IP experiment was conducted to verify whether CtBP2 and EGR-1 can interact with each other. The results of the Co-IP experiment demonstrated that EGR-1 protein could be detected by WB when pulled down with an anti-CtBP2 antibody. Similarly, when pulled down with an anti-EGR-1 antibody, CtBP2 protein was also detectable, further indicating that these proteins can interact (Figure 4C,D). The above results showed that CtBP2 and EGR-1 can combine with each other, and interference with CtBP2 inhibited the expression of EGR-1.

3.5 | Interfering With EGR1 Inhibited the Proliferation and Cycle Acceleration of DLBCL Cells and Promotes Apoptosis

To further explore the role of EGR-1 in DLBCL cells, we employed siRNA-EGR1 to silence EGR1 expression and evaluate the effects of EGR1 interference on cellular behavior. By comparing the transfection efficiency of different si-RNAs, we determined that siRNA-EGR1-1 exhibited the most significant effect, leading us to select this si-RNA for subsequent experiments (Figure 5A). The results from CCK-8 and EdU staining indicated that EGR1 inhibited the proliferation of DLBCL cells (Figure 5B,C). After transfection with siRNA-EGR1, the distribution of cells in the G1 phase increased, while the proportion in the S phase decreased, which indicated that the S phase of the cells was blocked (Figure 5D). In addition, through comparative analysis, we found that interference with EGR1 upregulated the apoptosis rate (Figure 5E) and inhibited the expression of Bcl-2 (an antiapoptotic protein), while promoting the expression of Bax and cleaved caspase-3 (proapoptotic proteins) (Figure 5F). This ultimately enhanced the apoptosis of DLBCL cells. However, the expression of CtBP2 remained unchanged after interfering with EGR-1, indicating that EGR1 could not regulate CtBP2 and is positioned downstream of CtBP2.

3.6 | Interfere With CtBP2 Inhibited the Proliferation and Cycle Acceleration of DLBCL Cells and Promoted Apoptosis Through EGR1

We have proved that EGR1 is located downstream of CtBP2 and is regulated by it. Subsequently, we constructed an overexpression plasmid for EGR1. Following successful transfection, the expression of EGR1 in the cells was significantly enhanced (Figure 6A). Through comparative analysis, we found that silencing CtBP2 inhibited the proliferation and cycle acceleration of DLBCL cells while promoting apoptosis. However, after further overexpression of EGR1, the effects of siRNA-CtBP2 were reversed: the proliferation of DLBCL cells increased again (Figure 6B,C), the distribution of cells in the G1 phase decreased while that in the S phase increased, leading to an accelerated cell cycle (Figure 6D). In addition, the level of the protein Bcl-2 increased while the expression of the proteins Bax and cleaved caspase-3 decreased, thereby inhibiting apoptosis in DLBCL cells once more (Figure 6E,F). The above experimental results suggested that interference with CtBP2 inhibited the proliferation and the acceleration of the cell cycle in DLBCL cells and promoted apoptosis through EGR1.

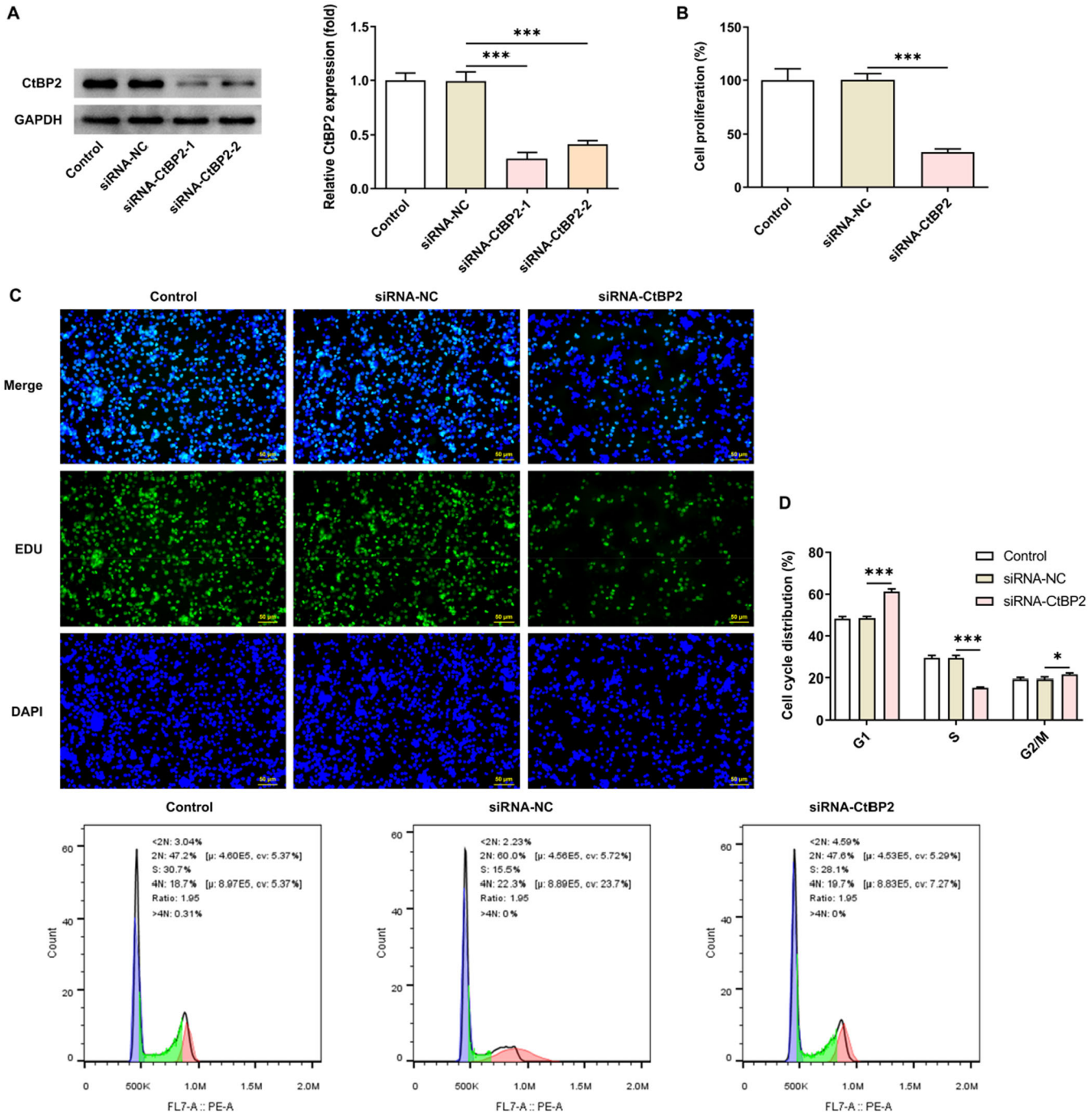

FIGURE 2 | Interference with CtBP2 inhibited the proliferation and cycle acceleration of DLBCL cells. The CtBP2 in cells was silenced through siRNA transfection. (A) The detection of the interference level of CtBP2 was performed by western blot. (B) Cell proliferation was detected by CCK8. (C) Cell proliferation was observed by EdU staining. (D) The cell cycle was detected by flow cytometry. *$p < 0.05$, ***$p < 0.001$. DLBCL, diffuse large B-cell lymphoma.

3.7 | EGR1 May Be Involved in the Regulation of the Wnt/β-catenin Signaling Pathway by CtBP2

Some studies have indicated that CtBP2 can participate in cancer progression through the Wnt/β-catenin signaling pathway. Therefore, we further investigated whether interfering with CtBP2 could impact the Wnt/β-catenin signaling pathway via EGR1 in DLBCL cells. Using WB analysis, we observed that the expression of the DKK1 protein increased while the levels of β-catenin and c-Myc proteins decreased after CtBP2 interference. However, upon further overexpression of EGR1, the effects of siRNA-CtBP2 were reversed: the expression of DKK1 protein was inhibited while the levels of β-catenin and c-Myc proteins increased (Figure 7). This suggested that CtBP2 may influence the expression of the Wnt/β-catenin signaling pathway through EGR1. Consequently, we judged that EGR1 could be one of the factors involved in the regulation of the Wnt/β-catenin signaling pathway by CtBP2.

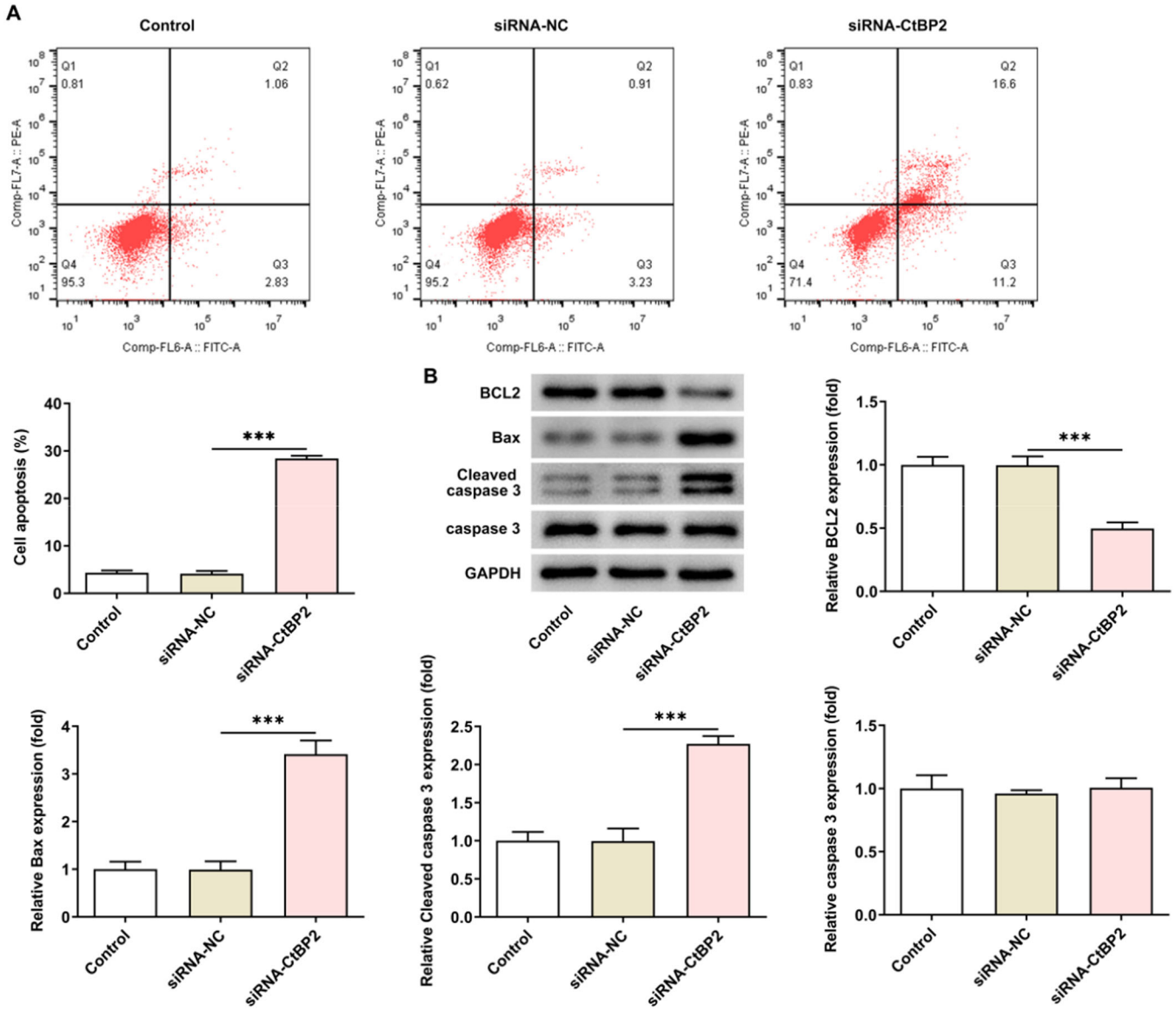

FIGURE 3 | Interference with CtBP2 promoted apoptosis of DLBCL cells. The CtBP2 in cells was silenced through siRNA transfection. (A) The level of apoptosis was detected by flow cytometry. (B) The expressions of Bcl-2, Bax, and caspase-3 (cleaved) in cells were detected by western blot. ***$p < 0.001$.

4 | Discussion

DLBCL is the most prevalent form of non-Hodgkin's lymphoma (NHL), accounting for approximately 30%–40% of all NHL cases [14]. This type of lymphoma is highly aggressive, with rapid disease progression [15, 16]. Although the currently recommended R-CHOP therapy has significantly improved the overall survival rate for DLBCL, 30%–40% of cases still experience relapse or exhibit resistance to drugs after treatment [17]. Therefore, there is an urgent need to identify new biomarkers that are involved in the onset and progression of DLBCL.

The vertebrate CtBP protein family primarily consists of CtBP1 and CtBP2, which serve various functions in development and tumorigenesis. These proteins are closely associated with the onset, progression, and metastasis of tumors [5]. CtBP1 and CtBP2 are upregulated in numerous tumor tissues, including those from breast cancer, ovarian cancer, colorectal cancer, gastric cancer, and melanoma, and their expression is linked to poor prognoses in these cancers [18–20]. However, it remains unclear whether CTBP2 can be involved in the malignant process of DLBCL. Firstly, we compared the expression levels of CtBP2 between normal cells and DLBCL cell line, and found that the expression of CtBP2 in DLBCL cell line was significantly enhanced. Then, we constructed an siRNA-CtBP2 interference plasmid to inhibit CtBP2 expression and conducted further experiments. The results from the CCK-8 assay and EdU staining indicated a decrease in cell proliferation and a slowdown in the cell cycle after interfering with CtBP2. The expression level of the antiapoptotic protein Bcl-2 decreased, while the levels of proapoptotic proteins Bax and cleaved caspase-3 increased significantly, which showed that interference with CtBP2 also promoted apoptosis in DLBCL cells.

Literature research shows that CtBP2 plays a biological role by binding to target proteins. The CtBP2-PCIF1 complex regulates

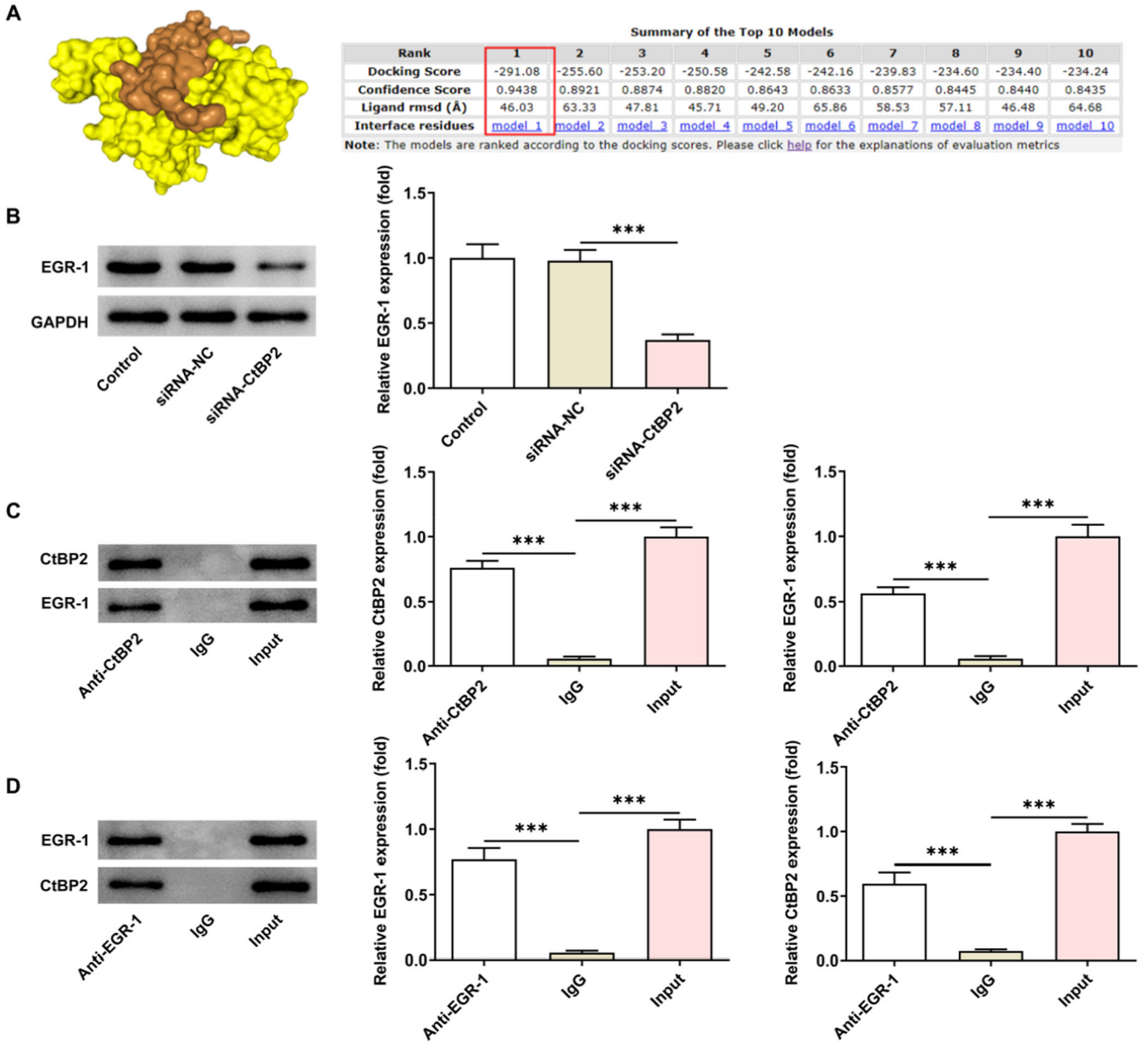

FIGURE 4 | Interference with CtBP2 inhibited the expression of EGR-1. The HDOCK server was utilized to predict the combination of CtBP2 and EGR-1. (B) The expression of EGR-1 was measured by western blot. (C, D) Co-IP assay was conducted to confirm the interaction between CtBP2 and EGR-1. ***$p < 0.001$.

the modification of m6Am mRNA and promotes the progression of head and neck squamous cell carcinoma [21]. Additionally, CtBP2 offers protection against oxidative stress through its interactions with NRF1 and NRF2 [22]. Furthermore, the interaction between CtBP2 and ZEB2 can regulate the expression of LEF1 in acute lymphoblastic lymphoma [23]. Studies have demonstrated that the expression of EGR1 is elevated in DLBCL, suggesting its role as a potential oncogene that promotes the proliferation of DLBCL cells [12], and CtBP2 has been shown to interact with EGR1 [10]. We utilized the HDOCK server to predict the interaction between CtBP2 and EGR-1, and the results indicated a strong binding affinity between the two proteins. Furthermore, Co-IP experiments confirmed that CtBP2 and EGR-1 can co-precipitate, providing further evidence of their robust interaction. After transfection with siRNA-CtBP2, the expression of the EGR-1 protein in the cells decreased significantly, indicating that the interference with CtBP2 inhibited the expression of EGR-1. To further explore the role of EGR-1 in DLBCL cells, we employed siRNA-EGR1 to silence EGR1 in the cells. Our findings revealed that the interference with EGR1 inhibited the proliferation of DLBCL cells and blocked the S phase of the cell cycle. Additionally, the interference with EGR1 reduced the expression of the Bcl-2 protein while promoting the expression of Bax and cleaved caspase-3 proteins, thereby enhancing the apoptosis of DLBCL cells. However, the expression of CtBP2 remained unchanged after the interference with EGR-1, indicating that EGR1 is positioned downstream of CtBP2 and is regulated by it.

We further overexpressed EGR1 in the cells. Through comparative analysis, we found that silencing CtBP2 inhibited the proliferation and cycle acceleration of DLBCL

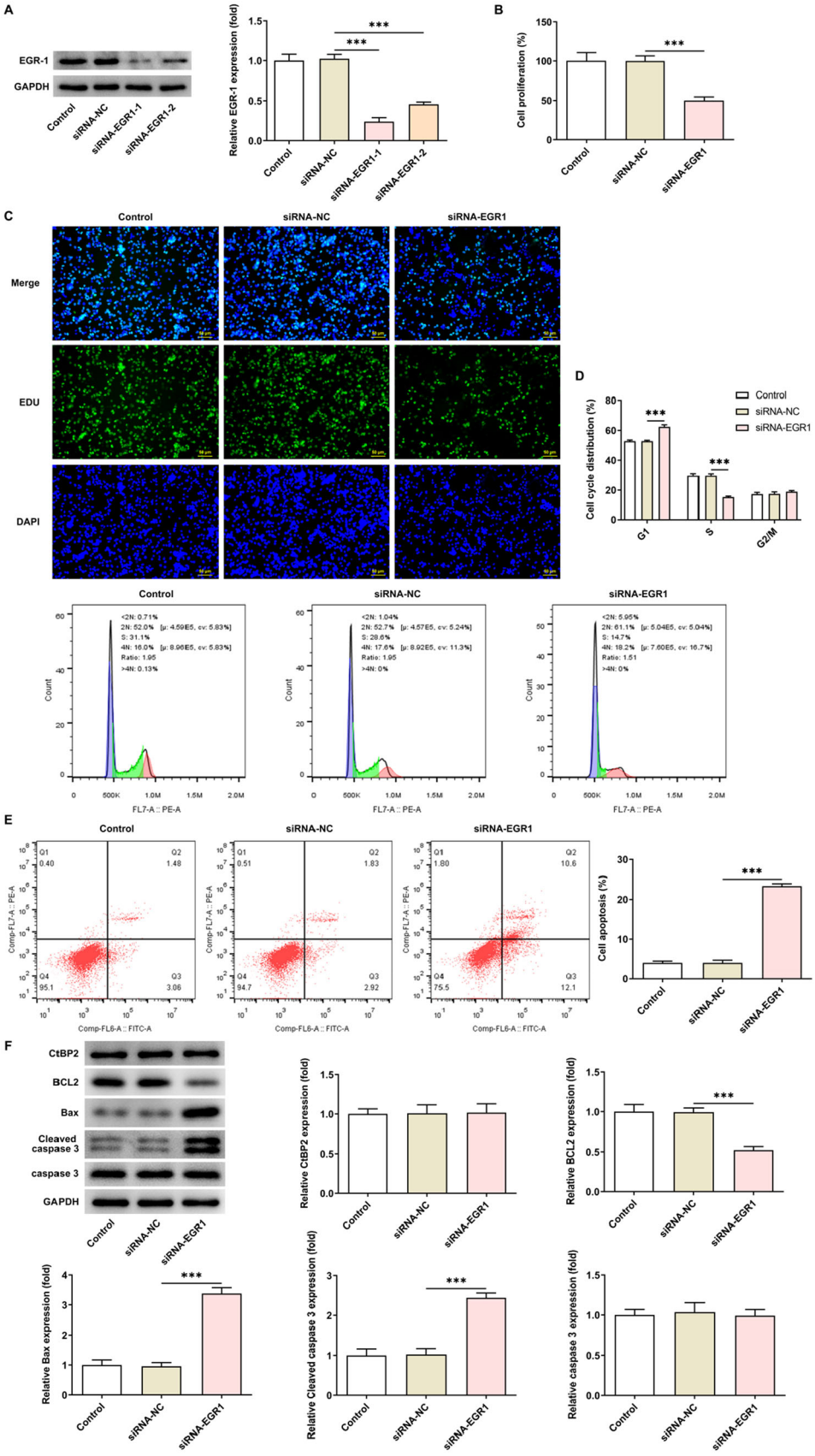

FIGURE 5 | Interfering with EGR1 inhibited the proliferation and cycle acceleration of DLBCL cells and promotes apoptosis. The EGR1 gene in cells was silenced through siRNA transfection. (A) The interference level of EGR1 was measured by western blot analysis. (B) Cell proliferation was detected by CCK8 assay. (C) EdU staining was performed to evaluate the level of cell proliferation; Flow cytometry was conducted to analyze the cell cycle (D) and the level of apoptosis (E). (F) The expressions of CtBP2 and apoptosis-related proteins were detected by western blot. ***$p < 0.001$.

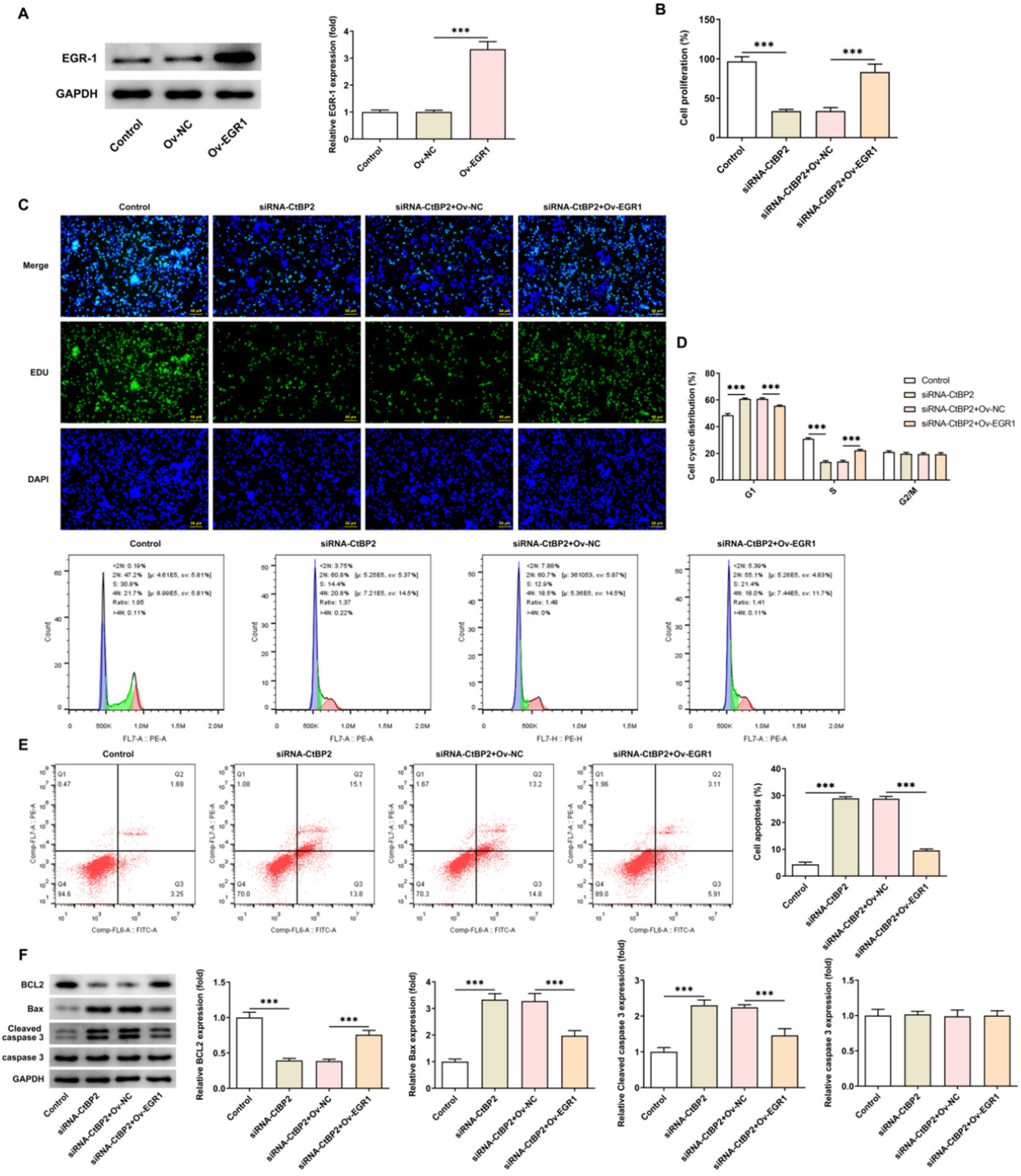

FIGURE 6 | Interfere with CtBP2 inhibited the proliferation and cycle acceleration of DLBCL cells and promoted apoptosis through EGR1. The cells were transfected with an Ov-EGR1 overexpression plasmid. (A) The overexpression level of EGR1 was assessed using Western blot analysis. (B) Cell proliferation was evaluated by CCK-8 assay. (C) Cell proliferation was measured through EdU staining; Flow cytometry was performed to analyze the cell cycle (D) and the level of apoptosis (E). (F) The expression of apoptosis-related proteins was detected by western blot assay. ***$p < 0.001$.

cells while promoting apoptosis. However, after the subsequent overexpression of EGR1, the effects of siRNA-CtBP2 were reversed: the proliferation of DLBCL cells increased, and the cell cycle was accelerated once again. Additionally, the expression of the protein Bcl-2 increased while the expression of the proteins Bax and caspase-3 decreased, and the apoptosis in DLBCL cells was inhibited again. This indicated that interference with CtBP2 inhibited the proliferation and

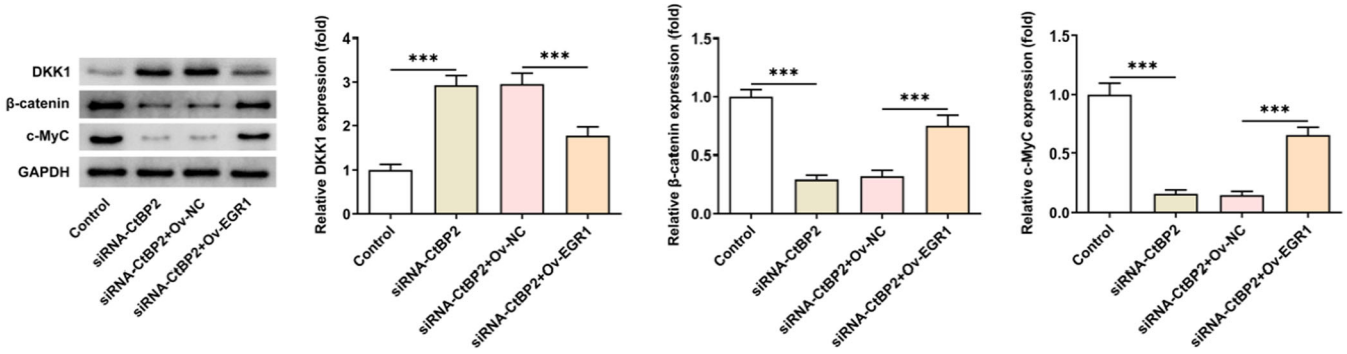

FIGURE 7 | EGR1 may be involved in the regulation of the Wnt/β-catenin signaling pathway by CtBP2. The expressions of Wnt/β-catenin signaling-related proteins DKK1, β-catenin and c-MyC were detected by western blot. ***$p < 0.001$.

the cycle acceleration in DLBCL cells and promotes apoptosis through EGR1.

Wnt signaling pathway is a crucial pathway involved in both physiological and pathological processes within cells. It regulates cell proliferation, differentiation, metastasis, and apoptosis, and is typically not activated in normal adults. The Wnt/β-catenin signaling pathway is prevalent in human cancers and animal experimental cancer models [24, 25]. Abnormal expression of the Wnt/β-catenin signaling pathway has been identified in triple-negative breast cancer [26], cholangiocarcinoma (CCA) [27], colorectal cancer [28], liver cancer (HCC) [29], non-small cell lung cancer (NSCLC) [30, 31] and other tumors. CtBP2 promotes the progression of esophageal squamous cell carcinoma [9] and the proliferation of prostate cancer cells [32] through the Wnt/β-catenin pathway.

In addition, some studies have found that CtBP2 can participate in the progression of NSCLC through the Wnt/β-catenin signaling pathway [8]. Therefore, we further explored whether the interference of CtBP2 affects the Wnt/β-catenin signaling pathway via EGR1 in DLBCL cells. First, we assessed the expression of proteins associated with the Wnt/β-catenin pathway. WB results indicated that the expression of DKK1 protein increased while the expression of β-catenin and c-Myc proteins decreased following CtBP2 interference. However, upon further overexpression of EGR1, the effects of siRNA-CtBP2 were reversed, which indicated that CtBP2 may influence Wnt/β-catenin signaling through EGR1. Thus, EGR1 may be one of the factors involved in the regulation of the Wnt/β-catenin signaling pathway by CtBP2.

5 | Conclusion

In summary, this study demonstrated that EGR1 may be one of the key contributors involved in the regulation of Wnt/β-catenin signaling by CtBP2 to affect both the proliferation and apoptosis in DLBCL cells. This discovery may offer a new insights for identifying biomarkers associated with the onset and progression of DLBCL, as well as strategies for inhibiting its advancement.

Author Contributions

Jianfang Dong: writing – original draft. **Lihua Li:** formal analysis. **Xuefei Zhang and Xijing Yin:** visualization. **Zucong Chen:** writing – review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data will be made available from the corresponding author upon reasonable request.

References

1. J. O. Armitage, "How I Treat Patients With Diffuse Large B-Cell Lymphoma," *Blood* 110, no. 1 (2007): 29–36, https://doi.org/10.1182/blood-2007-01-041871.

2. N. Howlader, A. B. Mariotto, C. Besson, et al., "Cancer-Specific Mortality, Cure Fraction, and Noncancer Causes of Death Among Diffuse Large B-Cell Lymphoma Patients in the Immunochemotherapy Era," *Cancer* 123, no. 17 (2017): 3326–3334, https://doi.org/10.1002/cncr.30739.

3. D. Chihara, J. R. Westin, Y. Oki, et al., "Management Strategies and Outcomes for Very Elderly Patients With Diffuse Large B-Cell Lymphoma," *Cancer* 122, no. 20 (2016): 3145–3151, https://doi.org/10.1002/cncr.30173.

4. M. A. Blevins, M. Huang, and R. Zhao, "The Role of CtBP1 in Oncogenic Processes and Its Potential as a Therapeutic Target," *Molecular Cancer Therapeutics* 16, no. 6 (2017): 981–990, https://doi.org/10.1158/1535-7163.mct-16-0592.

5. M. M. Dcona, B. L. Morris, K. C. Ellis, and S. R. Grossman, "CtBP-An Emerging Oncogene and Novel Small Molecule Drug Target: Advances in the Understanding of Its Oncogenic Action and Identification of Therapeutic Inhibitors," *Cancer Biology & Therapy* 18, no. 6 (2017): 379–391, https://doi.org/10.1080/15384047.2017.1323586.

6. P. Wang, B. Yu, C. Wang, and S. Zhou, "C-Terminal of E1A Binding Protein 2 Promotes the Malignancy of Osteosarcoma Cells via JAK1/Stat3 Signaling," *Journal of Cell Communication and Signaling* 14, no. 1 (2020): 67–76, https://doi.org/10.1007/s12079-019-00523-9.

7. L. Chen, L. Wang, J. Qin, and D. S. Wei, "CtBP2 Interacts With ZBTB18 to Promote Malignancy of Glioblastoma," *Life Sciences* 262 (2020): 118477, https://doi.org/10.1016/j.lfs.2020.118477.

8. D. P. Wang, L. L. Gu, Q. Xue, H. Chen, and G. X. Mao, "CtBP2 Promotes Proliferation and Reduces Drug Sensitivity in Non-Small Cell Lung Cancer via the Wnt/β-Catenin Pathway," *Neoplasma* 65, no. 6 (2018): 888–897, https://doi.org/10.4149/neo_2018_171220N828.

9. Q. Ju, M. Jiang, W. Huang, Q. Yang, Z. Luo, and H. Shi, "CtBP2 Interacts With TGIF to Promote the Progression of Esophageal Squamous Cell Cancer Through the Wnt/β-catenin Pathway," *Oncology Reports* 47, no. 2 (2022): 29, https://doi.org/10.3892/or.2021.8240.

10. Y. Wang, C. Qin, B. Zhao, et al., "EGR1 Induces EMT in Pancreatic Cancer via a P300/SNAI2 Pathway," *Journal of Translational Medicine* 21, no. 1 (2023): 201, https://doi.org/10.1186/s12967-023-04043-4.

11. J. Zhao, H. Li, and M. Yuan, "EGR1 Promotes Stemness and Predicts a Poor Outcome of Uterine Cervical Cancer by Inducing SOX9 Expression," *Genes & Genomics* 43, no. 5 (2021): 459–470, https://doi.org/10.1007/s13258-021-01064-5.

12. S. Kimpara, L. Lu, N. M. Hoang, et al., "EGR1 Addiction in Diffuse Large B-Cell Lymphoma," *Molecular Cancer Research* 19, no. 8 (2021): 1258–1269, https://doi.org/10.1158/1541-7786.mcr-21-0267.

13. T. Lei, X. Zhu, K. Zhu, F. Jia, and S. Li, "EGR1-Induced Upregulation of lncRNA FOXD2-AS1 Promotes the Progression of Hepatocellular Carcinoma via Epigenetically Silencing DKK1 and Activating Wnt/β-Catenin Signaling Pathway," *Cancer Biology & Therapy* 20, no. 7 (2019): 1007–1016, https://doi.org/10.1080/15384047.2019.1595276.

14. H. Mahmood, M. Habib, W. Aslam, et al., "Clinicopathological Spectrum of Diffuse Large B Cell Lymphoma: A Study Targeting Population Yet Unexplored in Pakistan," *BMC Research Notes* 14, no. 1 (2021): 354, https://doi.org/10.1186/s13104-021-05768-5.

15. H. Takahashi, N. Tomita, M. Yokoyama, et al., "Prognostic Impact of Extranodal Involvement in Diffuse Large B-Cell Lymphoma in the Rituximab Era," *Cancer* 118, no. 17 (2012): 4166–4172, https://doi.org/10.1002/cncr.27381.

16. T. A. Ollila and A. J. Olszewski, "Extranodal Diffuse Large B Cell Lymphoma: Molecular Features, Prognosis, and Risk of Central Nervous System Recurrence," *Current Treatment Options in Oncology* 19, no. 8 (2018): 38, https://doi.org/10.1007/s11864-018-0555-8.

17. A. K. Höhn, C. E. Brambs, G. G. R. Hiller, D. May, E. Schmoeckel, and L. C. Horn, "2020 WHO Classification of Female Genital Tumors," *Geburtshilfe und Frauenheilkunde* 81, no. 10 (2021): 1145–1153, https://doi.org/10.1055/a-1545-4279.

18. J. S. Byun and K. Gardner, "C-Terminal Binding Protein: A Molecular Link Between Metabolic Imbalance and Epigenetic Regulation in Breast Cancer," *International Journal of Cell Biology* 2013 (2013): 647975, https://doi.org/10.1155/2013/647975.

19. M. M. Dcona, P. K. Damle, F. Zarate-Perez, et al., "Active-Site Tryptophan, the Target of Antineoplastic C-Terminal Binding Protein Inhibitors, Mediates Inhibitor Disruption of CtBP Oligomerization and Transcription Coregulatory Activities," *Molecular Pharmacology* 96, no. 1 (2019): 99–108, https://doi.org/10.1124/mol.118.114363.

20. C. Wang, M. Wang, B. Xing, et al., "C-Terminal of E1A Binding Protein 1 Enhances the Migration of Gastric Epithelial Cells and Has a Clinicopathologic Significance in Human Gastric Carcinoma," *OncoTargets and Therapy* 12 (2019): 5189–5200, https://doi.org/10.2147/ott.s203479.

21. K. Li, J. Chen, C. Zhang, et al., "The CTBP2-PCIF1 Complex Regulates m6Am Modification of MRNA in Head and Neck Squamous Cell Carcinoma," *Journal of Clinical Investigation* 133, no. 20 (2023): 1–17, https://doi.org/10.1172/jci170173.

22. K. Kainoh, R. Takano, M. Sekiya, et al., "CtBP2 Confers Protection Against Oxidative Stress Through Interactions With NRF1 and NRF2," *Biochemical and Biophysical Research Communications* 562 (2021): 146–153, https://doi.org/10.1016/j.bbrc.2021.05.069.

23. C. J. Wang, M. Z. Jia, L. P. Deng, et al., "Interaction Between CASP8AP2 and ZEB2-CtBP2 Regulates the Expression of LEF1," *Pediatric Hematology and Oncology* 39, no. 6 (2022): 549–560, https://doi.org/10.1080/08880018.2022.2033369.

24. F. Yu, C. Yu, F. Li, et al., "Wnt/β-catenin Signaling in Cancers and Targeted Therapies," *Signal Transduction and Targeted Therapy* 6, no. 1 (2021): 307, https://doi.org/10.1038/s41392-021-00701-5.

25. X. Wen, Y. Wu, A. Awadasseid, Y. Tanaka, and W. Zhang, "New Advances in Canonical Wnt/β-Catenin Signaling in Cancer," *Cancer Management and Research* 12 (2020): 6987–6998, https://doi.org/10.2147/cmar.s258645.

26. W. Xie, Y. Zhang, Y. He, et al., "A Novel Recombinant Human Frizzled-7 Protein Exhibits Anti-Tumor Activity Against Triple Negative Breast Cancer via Abating Wnt/β-Catenin Pathway," *International Journal of Biochemistry & Cell Biology* 103 (2018): 45–55, https://doi.org/10.1016/j.biocel.2018.08.004.

27. G. F. Zhang, L. Qiu, S. L. Yang, J. C. Wu, and T. J. Liu, "Wnt/β-catenin Signaling as an Emerging Potential Key Pharmacological Target in Cholangiocarcinoma," *Bioscience Reports* 40, no. 3 (2020): BSR20193353, https://doi.org/10.1042/bsr20193353.

28. X. Cheng, X. Xu, D. Chen, F. Zhao, and W. Wang, "Therapeutic Potential of Targeting the Wnt/β-catenin Signaling Pathway in Colorectal Cancer," *Biomedicine & Pharmacotherapy* 110 (2019): 473–481, https://doi.org/10.1016/j.biopha.2018.11.082.

29. V. Vilchez, "Targeting Wnt/β-catenin Pathway in Hepatocellular Carcinoma Treatment," *World Journal of Gastroenterology* 22, no. 2 (2016): 823–832, https://doi.org/10.3748/wjg.v22.i2.823.

30. R. Wang, J. Liu, K. Li, et al., "An SETD1A/Wnt/β-catenin Feedback Loop Promotes NSCLC Development," *Journal of Experimental & Clinical Cancer Research* 40, no. 1 (2021): 318, https://doi.org/10.1186/s13046-021-02119-x.

31. L. Qu, Y. Tian, F. Wang, and Z. Li, "NOVA1 Promotes NSCLC Proliferation and Invasion by Activating Wnt/β-Catenin Signaling," *BMC Cancer* 22, no. 1 (2022): 1091, https://doi.org/10.1186/s12885-022-10164-8.

32. C. Zhang, C. Gao, Y. Xu, and Z. Zhang, "CtBP2 Could Promote Prostate Cancer Cell Proliferation Through c-Myc Signaling," *Gene* 546, no. 1 (2014): 73–79, https://doi.org/10.1016/j.gene.2014.05.032.