Overexpression of miR-222-3p promotes proliferation and inhibits apoptosis in diffuse large B-cell lymphoma cells via suppressing PPP2R2A

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Abstract

Background This study aimed to investigate the mechanism of microRNA-222-3p (miR-222-3p) on the progression of diffuse large B-cell lymphoma (DLBCL) cells.Methods DLBCL tissue was isolated from DLBCL patients during surgery. OCI-LY10 and U2932 cells were cultured. Then, qRT-PCR, Western blot, luciferase reporter gene assay, RNA pull-down assay, MTT assay, colony formation analysis, flow cytometry as well as Transwell assay were used to observe the effect of miR-222-3p on proliferation, migration, invasion and apoptosis of DLBCL cells. Furthermore, the tumor growth affected by miR-222-3p was further investigated based on animal experiment.Results Compared with the control group, the expression level of miR-222-3p was up-regulated in DLBCL group. The luciferase reporter gene and RNA pull down assay showed that PPP2R2A 3'-untranslated region (3'-UTR) carried the directly binding site of miR-222-3p. Furthermore, MTT assay, colony formation, qRT-PCR and Western blot showed that miR-222-3p promoted the DLBCL cell proliferation and invasion, and inhibited apoptosis. Finally, the mice experiment showed that miR-222-3p mimics inhibited PPP2R2A expression and promoted tumor growth in vivo.Conclusions Upregulation of miR-222-3p might take part in the progression of DLBCL by suppressing PPP2R2A expression. Furthermore, miR-222-3p promoted the DLBCL cell proliferation and invasion, and inhibited apoptosis.

Background

As an extremely invasive non-Hodgkin's lymphoma, diffuse large B-cell lymphoma (DLBCL) accounts for about 30% - 40% of all non-Hodgkin's lymphoma cases. [1] The initial response rate of DLBCL reaches 90%, but the 5-year recurrence rate is as high as 40%. [2] Drug resistance in treatment also greatly plagued clinicians and patients. About one-third of DLBCL patients die of relapse or drug resistance. [3] The unclear pathogenesis hinders further progress in the treatment of DLBCL.

In recent years, some molecular pathogenesis of DLBCL has been initially discovered.[4] In addition to coding genes, several non-coding genes are also considered to be one of the vital targets regulating the progression of DLBCL, especially microRNAs (miRNAs).[5] miRNAs are key regulators in tumorigenesis and development during last decade.[6] The important role of these miRNAs in the progression of DLBCL have also mentioned in previous studies. [7,8] As a member of miRNAs, miR-222 has been proved to participate in the process of multiple diseases such as breast cancer, nasopharyngeal carcinoma and colorectal cancer. [9-11] A previous study indicates that the intestinal inflammation can be aggravated by up-regulation of miR-222 during the disease progression. [12] Importantly, there is a strong relationship between miR-222 and the development of DLBCL. [13] Based on a miRNAs expression profile, a previous study indicates that miR-222 is a potential biomarker for EBV-positive DLBCL. [14] Actually, recent study shows that the biological function of miR-222 in disease is commonly realized by targeting certain genes such as Protein Phosphatase 2 Regulatory Subunit Balpha (PPP2R2A). [15] Dong et al. showed that up-regulation of miR-222 plays a vital role in the process of liver fibrosis in biliary atresia by targeting

PPP2R2A. [16] Although sporadic research has been focused on the biological function of miR-222 in diseases, the detail molecular mechanism of miR-222 during the progression of DLBCL is still unclear.

In this study, DLBCL tissue were isolated from DLBCL patients. Moreover, the effect of miR-222 on proliferation, migration, invasion and apoptosis of DLBCL cells was detected. Finally, the tumor growth affected by miR-222 was investigated based on animal experiment. We hoped to explore the detail molecular mechanism of miR-222 in the process of DLBCL.

Methods

Patients and sample collection

A total of 74 cases of initial diagnosis of DLBCL admitted to our hospital from February 2012 to November 2013 were enrolled in current study. No previous chemotherapy, radiation or other biological treatments were considered as the inclusion criteria. Meanwhile, the other types of lymphoma, and DLBCL combined with other diseases were considered as the exclusion criteria. Moreover, a total of 26 patients with pathological diagnosis of reactive lymphoid hyperplasia were selected as controls. The specimens were excised during surgery, and then stored in liquid nitrogen at 80°C until RNA extraction. The overall survival (OS) was defined as the date from enrollment to death. This study has been approved by the ethics committee of Shouguang People's Hospital of Shandong Province. All patients signed a written informed consent form prior to the study.

Cell culture

Human normal B cell immortalized cell line (HMy2.CIR), DLBCL cell line, central B cell-like (GCB) OCI-Ly19 and SU-DHL-4 cells, activated B cell-like (ABC) OCI-LY10 and U2932 cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HMy2.CIR was cultured in IMDM (Gibco, USA) medium containing 10% FBS and 1% penicillin-streptomycin (P/S). U2932 and SU-DHL-4 cells were cultured in RPMI 1640 medium (Gibco, USA) medium containing 10% FBS and 1% P/S. OCI-LY10 and OCI-Ly19 cells were cultured in IMDM (Gibco, USA) medium containing 20% FBS and 1% penicillin-streptomycin (P/S). All cells were cultured in a humidified humidity incubator with 5% CO_2 at 37 °C.

Cell transfection and grouping

OCI-LY10 and U2932 cells in good growth condition were collected and seeded in 6-well plates (5×10^5 cells/well). miR-222-3p mimics, miR-222-3p inhibitors, mimics negative control (NC), inhibitors NC, pcDNA3.1-NC and pcDNA3.1-PPP2R2A (Shanghai Jima Company) (15 μl for each) were dissolved in 250 ml medium and mixed evenly to obtain A solution. Meanwhile, a total of 5 ml EntransterTM-R transfection reagent (Engreen Biosystem) was added to 250 ml culture medium and mixed evenly to obtain B liquid. Then, the solution A and B were mixed evenly and incubated in incubator for 48 h. According to different treatments, all cells were divided into miR-222-3p mimics group, mimics NC group,

miR-222-3p inhibitors group and inhibitors NC group, mimics NC + pcDNA3.1-NC group, mimics + pcDNA3.1-NC group, mimics NC + pcDNA3.1-PPP2R2A group and mimics + pcDNA3.1-PPP2R2A group. The untransfected cells were named as Blank group.

qRT-PCR

The expression level of miR-222-3p was detected by qRT-PCR. Briefly, cells cultured for 48 h were collected to extract total RNA by using Trizol method, and reverse-transcribed using random hexamer primers (invitrogen, San Diego, USA) in accordance with manufacturers' instructions. qRT-PCR was performed on ABI PRISMR 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The PCR program included 40 cycles of 95°C for 3 min, 95°C for 10 s and 55°C for 30 s. Data was analyzed by the $2^{-\Delta\Delta Ct}$ method [17], and all oligonucleotide primers were designed and synthesized by Biotechnology Bioengineering Co., Ltd. (Shanghai). The primer sequences for samples and internal references were listed in Table 1.

Luciferase reporter gene assay

The target sites for miR-222-3p were determined by Target Scan (http://www.targetscan.org/), and the wild-type (PPP2R2A-WT) or mutant (PPP2R2A-MUT) of miR-222-3p were designed according to the predicted results. The miR-222-3p mutant sequence and the wild sequence fragment were cloned to the pGL3 luciferase control reporting vector (Promega, USA). Then, the EHK-293T cells (ATCC) were seeded into 24-well plates (5×10^5 cells/well) and co-transformed with PPP2R2A-WT (or PPP2R2A-MUT) and miR-222-3p mimic (or negative control) using Lipofectamine 2000 (Thermo Fisher Scientific). After transfection for 48 h, luciferase assay was determined by dual luciferase reporter assay kit (Promega).

RNA pull-down assay

miR-222-3p-Wt, miR-222-3p-Mut and miR-NC were transcribed using the TranscriptAid T7 High Yield Transcription Kit (ThermoFisher Scientific, Waltham, MA, USA). The Biotin RNA labeling cocktail (Roche Diagnostics, Indianapolis, IN, USA) was used to synthesize Bio-miR-222-3p-Wt, Bio miR-222-3p-Mut and Bio-miR-NC. Furthermore, a total of 50 pmol of biotinylated RNA was mixed with 200 μg of cell lysate (OCI-LY10 or U2932 cells), and then incubated with 50 μl of streptavidin agarose (Invitrogen, Carlsbad, CA, USA) for 1 hour at 4 °C. Fiannly, the eluted proteins were measured using RT-qPCR.

MTT assay

Transfected cells in the logarithmic growth phase were seeded in 96-well plates (6×10^3 cells per well), and cultured in incubator (37°C, 5% CO_2) for 24-72h. MTT (5 mg/mL) was then added into each well with a volume of 20 μL. Culture was terminated after continuing incubation in the incubator for 4 h. Then, a total of 150 μl DMSO was added to each well to promote crystallization dissolution. The absorbance values of the wells at 0h, 24h, 48h and 72h were measured, followed by the construction of MTT plot (Y-axis: absorbance value; X-axis: interval time). The experiment was repeated for 3 times.

Clonal colony formation assay

Cells in each group were cultured for 48 hours after transfection, and then cells were washed with PBS and digested with 1% trypsin. All the cells were primarily added into 6-well plates (300 cells/well) with 2.5 mL medium in each well. Two weeks later, residual liquid in each well was discarded. After washed by PBS for twice, cells were fixed with 4 % paraformaldehyde solution and then stained with Swiss-Gimsa for 15 min. Then residual liquid in each well was discarded, all plates were dried at room temperature. Clones were counted automatically by using ImageJ (1.48V) software, and then photographed with an inverted phase contrast microscope (Olympus Ckx53). Cell colony formation rate was calculated as (number of colonies/total number of cells seeded) × 100%.

Flow cytometry

The effect of miR-222-3p mimics or inhibitors on OCI-LY10 and U2932 cells apoptosis was measured by Annexin V/FITC apoptosis detection kit (Kaiji Biotechnology Co., Ltd., China). Mixed solution consisting of 5 μL PI and 5 μL Annexin V/FITC was added to cells for 15 min incubation. Apoptosis was conducted by flow cytometry according to the procedure in the Annexin V-FITC/PI Assay Kit.

Transwell assay

The logarithmic growth phase of OCI-LY10 and U2932 cells wer inoculated into the Transwell chamber (Corning, USA), and the cell density of each group was adjusted to 2×10^5/mL with serum-free RPMI-1640 medium. A total of 200 μL samples was added to the upper chamber, while 400 μL of RPMI-1640 medium containing 20% FBS was added to the lower chamber (24-well plate). After 48 hours of culturation, the chamber was removed and the medium in the plate was aspirated, washed twice with PBS, fixed with 4% paraformaldehyde for 30 min, and stained with crystal violet for 20 min. Then room dried, and photographed under the microscope. Five random fields were used to count the number of transmembrane cells (mean and standard deviation) under a microscope (200 ×), and the number of cells passing through the cells was used to indicate the invasive ability.

Western blot analysis

Protein expression was measured by Western blot. Briefly, a total of 50 μg total protein was extracted by lysis buffer, followed by quantifying using a BCA kit (Nanjing Kaiji Biotech Co., Ltd.). Then samples was subjected to 10 % SDS-PAGE, and transferred onto PVDF membrane. Afterwards, the membrane was blocked with 5% skim milk in TBST solution. Subsequently, membrane was sequentially incubated with primary antibodies (Rabbit anti-human Bcl-2, Bax, PPP2R2A, 1:2000, abcam, UK) and the secondary antibodies (bs-0295G-HRP, 1:5000, Beijing Biosynthesis Biotechnology Co., Ltd., China). At last, blots were visualized by enhanced chemiluminescence Plus, and integrate optical density was measured by software Lab Works 4.5.

Tumor growth assay

A total of 18 SPF BALB/c nude mice (4-week-old) were purchased from SLACL laboratory animal center (Shanghai, China). Then, 0.1 ml of 1.0×10^7/ml OCI-LY10 cells from Blank, miR-222-3p mimics or mimics NC group, respectively were injected subcutaneously into the flank of nude mice (6 mice in each group). After transplantation, the tumor growth and tumor volume were observed every 7 days, and the final measurement was performed on the 56 th day. Tumor volume was estimated according to the following formula: $L \times W^2/2$ (where L represented the length, and W represented the width). At the end of the experiment (on day 56), mice were anesthetized (with CO_2) and killed. Tumor tissue was dissected and collected for further analysis. All animal studies were approved by the Animal Ethics Committee of Shouguang People's Hospital of Shandong Province and were conducted in compliance with animal-use guidelines established in Shandong, China.

Statistical analysis

All statistical analyses were performed using SPSS 21.0 statistical software. The results were presented in the form of mean ± standard deviation (SD). The data of two groups were analyzed by the Student t test. $P < 0.05$ was considered to be statistically significant.

After the study, all animals were euthanized. The right hand held the rat tail and pull it back, and the left thumb and forefinger pressed down firmly on the mouse head at the same time. The external force was used to dislocate the cervical spine of the mouse, and the spine and the brain were disconnected. This method can quickly lose consciousness and reduce pain of experimental animals, which is a commonly used method for euthanasia of small experimental animals.

Results

miR-222-3p was up-regulated in DLBCL

The expression levels of miR-222-3p in DLBCL patients, control group, HMy2.CIR, GBC and ABC type DLBCL were detected by qRT-PCR. The result showed that miR-222-3p expression levels in DLBCL patients were higher than those in the control group (Figure 1A). Kaplan-Meier analysis showed that the overall survival in patients with low miR-222-3p expression was significantly higher than that in patients with high miR-222-3p expression (Figure 1B). The expression levels of miR-222-3p in DLBCL cell lines (OCI-LY19, SU-DHL-4, OCI-LY10 and U2932) were higher than those in HMy2.CIR (Figure 1C). Since the miR-222-3p expression levels in ABC-type DLBCL was higher than those of GBC-type cells, the ABC-type DLBCL cell lines OCI-LY10 and U2932 were selected for further investigation. Furthermore, we studied the relationship between miR-222-3p expression and the clinicopathological parameters of DLBCL. As shown in Table 2, compared with patients of III/IV stage, extranodal invasion and IPI score with 3-5, the expression levles of miR-222-3p were significantly higher than those in patients of I/II stage, no extranodal invasion and IPI score with 0-2 (all $P < 0.05$), suggesting that the upregulation of miR-222-3p may be involved in human DLBCL tumorigenesis.

MiR-222-3p promoted proliferation and invasion in DLBCL cells

To investigate the effect of miR-222-3p on DLBCL cells proliferation and inviasion, we first detected its expression levels of miR-222-3p mimics and miR-222-3p inhibitor. We found that the expression levels of miR-222-3p in miR-222-3p mimics group were significantly higher than those in mimics NC group (all $P < 0.05$) in both OCI-LY10 and U2932 cells (Figure 2A). Meanwhile, miR-222-3p expression in miR-222-3p inhibitors group were significantly lower than those in inhibitors NC group (all $P < 0.05$) (Figure 2B). MTT assay showed that compared with mimics NC group, the proliferation of OCI-LY10 and U2932 cells were promoted in miR-222-3p mimics group (all $P < 0.05$). Meanwhile, compared with inhibitors NC group, the proliferation of OCI-LY10 and U2932 cells were inhibited in miR-222-3p inhibitors group (all $P < 0.05$) (Figure 2C).

The results of colony formation experiment in OCI-LY10 and U2932 cells showed that the number of cell clones in the miR-222-3p mimics group was significantly higher than that in the mimics NC group (all $P < 0.05$). Meanwhile, the number of cell clones in the miR-222-3p inhibitor group was significantly lower than that in the inhibitor NC group (all $P < 0.05$) (Figure 2D).

Transwell assay showed that the round translucent suspension cells were observed in all four groups and the invasive ability of OCI-LY10 and U2932 cells in miR-222-3p mimics group was higher than that in mimics NC group. Meanwhile, the invasive ability of cells in miR-222-3p inhibitors group was lower than that in inhibitors NC group (all $P<0.05$) (Figure 2E). All of these indicated that miR-222-3p could promote proliferation and invasion in OCI-LY10 and U2932 cells.

MiR-222-3p inhibited apoptosis in DLBCL cells

Flow cytometry was used to detect the effects of miR-222-3p mimics or inhibitors transfection on OCI-LY10 and U2932 cells apoptosis. The results showed that apoptotic rate of OCI-LY10 and U2932 cells transfected with miR-222-3p mimics was significantly lower than that transfected with mimics-NC (all $P < 0.05$). The apoptotic rate of OCI-LY10 and U2932 transfected with miR-222-3p inhibitors was significantly higher than that of transfected inhibitors-NC ($P < 0.05$) (Figure 3A).

Western blot was used to detect the expression of Bcl-2 and Bax in both OCI-LY10 and U2932 cells. The results showed that compared with mimics NC group, Bcl-2 and Bax was significantly up- and down-regulated respectively in miR-222-3p mimics group (all $P < 0.05$). Meanwhile, compared with inhibitors NC group, Bcl-2 and Bax was significantly down- and up-regulated in miR-222-3p inhibitors group ($P < 0.05$) (Figure 3B).

PPP2R2A was the target gene of miR-222-3p

Target Scan showed the binding site of miR-222-3p to the 3'-UTR of PPP2R2A (Figure 4A). The luciferase activity of miR-222-3p mimics and PPP2R2A wild-type recombinant vector transfection group were significantly decreased ($P < 0.05$) (Figure 4B). Moreover, RNA pull-down experiments further indicated that miR-222-3p binds directly to PPP2R2A (Figure 4C). The mRNA expression of PPP2R2A was decreased in OCI-LY10 and U2932 cells after transfection with miR-222-3p mimics compared with mimics NC group.

The expression of PPP2R2A mRNA in OCI-LY10 and U2932 cells increased after transfection of pcDNA3.1-PPP2R2A. Meanwhile, the co-transfection of miR-222-3p mimics and pcDNA3.1-PPP2R2A abrogated the down-regualtion effect of miR-222-3p mimics on PPP2R2A mRNA (Figure 4D). Moreover, western blot further verified that co-transfection of miR-222-3p mimicspc and pcDNA3.1-PPP2R2A regulated the expression of PPP2R2A (Figure 4E). The expression level of PPP2R2A in DLBCL patients was lower than that in the control group (Figure 4F). Furthermore, there was a negative correlation between miR-222-3p expression level and PPP2R2A expression level in DLBCL patients ($r = -0.6862$ $p < 0.0001$) (Figure 4G). The expression level of PPP2R2A in DLBCL cell lines (OCI-LY19, SU-DHL-4, OCI-LY10 and U2932) was lower than that in the HMy2.CIR (Figure 4H).

Overexpression of PPP2R2A regulated the effect of miR-222-3p on DLBCL proliferation and apoptosis

To research the biological function of miR-222-3p on DLBCL, MTT assay were performed and we found that miR-222-3p promoted the proliferation of OCI-LY10 and U2932 cells (Figure 5A). Overexpression of PPP2R2A could partially abrogated miR-222-3p-mediated effect on the proliferation of OCI-LY10 and U2932 cells (Figure 5A). . Moreover, compared with mimics NC + pcDNA3.1-NC group, the apoptotic rate of mimics NC + pcDNA3.1-NC group was significantly decreased. Compared with mimics NC + pcDNA3.1-NC group, the apoptotic rate of mimics NC + pcDNA3.1-PPP2R2A group was significantly increased (Figure 5B). Furthermore, compared with the mimics NC + pcDNA3.1-NC group, the Bcl-2 and Bax was significantly up- and down-regulated respectively in the mimics 222-3p mimics + pcDNA3.1-NC group. Compared with mimics NC + pcDNA3.1-NC group, Bcl-2 and Bax protein was significantly down- and up-regulated respectively in mimics NC + pcDNA3.1-PPP2R2A group (Figure 5C). All of these findings reveal that PPP2R2A functions as a sponge of miR-222-3p in DLBCL cells.

Mi-222-3p mimics promotes tumor growth in nude mice

To further determine the *in vivo* therapeutic efficacy of miR-222-3p in nude mice, xenograft tumor model was constructed. In mice experiment, the tumor volume was the largest on the 56th day in the microRNA-222-3p mimics group (Figure 6A). Similarly, on the 21th, 35th and 49th day, the tumor volume was also larger than that in the mimics NC group (Figure 6B). Moreover, the qRT-PCR showed that the overexpression of microRNA-222-3p inhibits the expression of PPP2R2A in tumors (Figure 6C) and protein expression (Figure 6D). These results suggest that microRNA-222-3p mimics inhibited PPP2R2A-induced xenograft growth in nude mice.

Discussion

DLBCL was a common malignant tumor with strong invasive ability. [18] Although some genes including miR-222 has been proved to be related with the development of DLBCL, [12] the detail mechanism of miR-222 in DLBCL progression is still unclear. In this study, compared with the control group, miR-222-3p was significantly up-regulated in DLBCL group. The luciferase reporter gene and RNA pull down assay showed that PPP2R2A 3’-untranslated region (3’-UTR) carried the directly binding site of miR-222-3p. Furthermore,

the MTT assay, colony formation, qRT-PCR and Western blot showed that miR-222-3p promoted the DLBCL cell proliferation and invasion, and inhibited apoptosis. Finally, the mice experiment showed that miR-222-3p mimics inhibited PPP2R2A expression and promoted tumor growth *in vivo*.

As a therapeutic target, miR-222 has been proved to be up-regulated in the development of various diseases. [19,20] A previous study shows the upregulation of miR-221/222 expression has been revealed in rheumatoid arthritis (RA) patients. [21] Noormohammad et al. indicated that miR-222 is overexpressed in both Helicobacter pylori-infected and noninfected gastric cancer patients. [22] This expression trend of miR-222 has also been proved in DLBCL based on a study of miRNA signature profile. [23] Garofalo et al. indicate that downregulation of miR-222 contributes to the enhanced tumorigenicity. [24] Gan et al. showed that downregulation of miR-222 enhances sensitivity of breast cancer cells to certain medicine such as tamoxifen. [25] Actually, the miR-222 overexpression promotes proliferation of tumor cells via certain target gene. [26] Interestingly, miR-222 overexpression may contribute to the development of disease by targeting PPP2R2A. [16] A previous study shows that the PPP2R2A deletions were recently linked to a subgroup of luminal breast carcinoma that exhibits poor survival. [27] Down-regulation of PPP2R2A is proved to inhibit homologous recombination DNA repair and predict tumor sensitivity to PARP inhibition. [28] Wang et al. showed that the levels of PPP2R2A was lower in giant cell tumor of bone tissues than that in normal tissues. [29] Importantly, a recent study indicates that the upregulation of miR-614 promotes proliferation and inhibits apoptosis in ovarian cancer by suppressing PPP2R2A expression, [30] which indicating an important role of miRNA-PPP2R2A regulation relation in tumor progression. In the current study, miR-222-3p and PPP2R2A was proved to be significantly up- and down-regulated respectively in DLBCL cells. Meanwhile, the luciferase reporter gene and RNA pull down assay showed that PPP2R2A 3'-UTR carried the directly binding site of miR-222-3p. Thus, we speculated that the upregulation of miR-222-3p might take part in the progression of DLBCL by suppressing PPP2R2A expression.

The expression of miRNAs is widely accepted to be pathogenetically involved in DLBCL. [31,32] MiR-222 is an important member of the miRNAs, which can affect multiple process of tumor cell including proliferation, differentiation, apoptosis, invasion and metastasis. [33] A previous study shows that the down-regulation of miR-222 restrain prostate cancer cell proliferation and migration. [34] Liu et al. indicated that miRNA-222 promoted liver cancer cell proliferation, migration, invasion and inhibits apoptosis. [33] Inhibition of miR-222-3p leads to the low activity of cells proliferation and invasion. [35] Moreover, previous study shows that miR-222 can induce cell apoptosis during the process of gastrointestinal stromal tumor. [36] This effect has also been proved in prostate cancer cells and neck squamous cell carcinoma cells. [37,38] In this study, the MTT assay, colony formation, qRT-PCR and Western blot showed that compared with the mimics NC group, the cell proliferation and invasion were enhanced in OCI-LY10 and U2932 cells, while the apoptosis was decreased in miR-222-3p mimics group. Thus, we speculated that miR-222-3p promoted the OCI-LY10 and U2932 cell proliferation and invasion, and inhibited apoptosis. However, there were some limitations in current study such as small sample size and lack of verification analysis. Thus,

a further verification study based on a large sample size is needed to confirm all speculations in this study.

Conclusions

In conclusion, upregulation of miR-222-3p might take part in the progression of DLBCL by suppressing PPP2R2A expression. Furthermore, miR-222-3p promoted the DLBCL cell proliferation and invasion, and inhibited apoptosis.

Declarations

Ethics approval and consent to participate: This study was conducted after obtaining Shouguang People's Hospital of Shandong Province's ethical committee approval and written informed consent from the patients.

Consent for publication: Not applicable.

Availability of data and material: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: SSS designed and analyzed the experiment, and was a major contributor in writing the manuscript. HW and MYJ performed the experiment. All authors read and approved the final manuscript.

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Figures

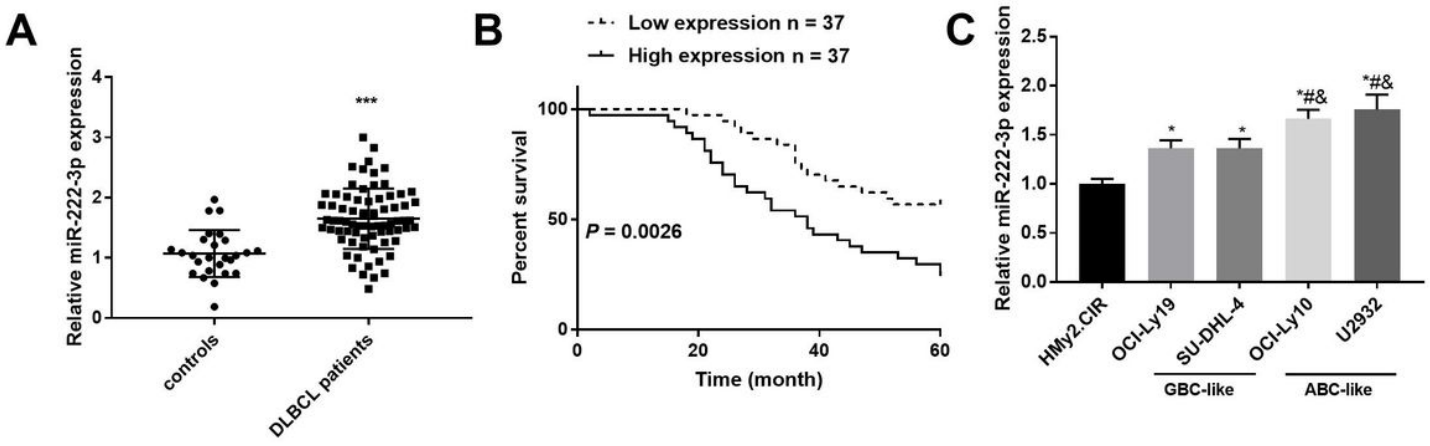

Figure 1

Expression levels of miR-222-3p in diffuse large B-cell lymphoma. A, the miR-222-3p levels in DLBCL and controls detected by qRT-PCR; compared with the control group, ***P<0.0001. B, Kaplan-Meier detection for the overall survival rate of patients with low expression and high expression of miR-222-3p; p=0.0026. C, the expression levels of miR-222-3p in DLBCL cell lines (OCI-LY19, SU-DHL-4, OCI-LY10 and U2932) and human normal B cell immortalized cell line (HMy2.CIR) detected by RT-PCR. Compared with HMy2.CIR, *P < 0.05; compared with OCI-LY19, #P<0.05; compared with SU-DHL-4, & P<0.05. Data were expressed as mean ± standard deviation. All experiments were repeated 3 times.

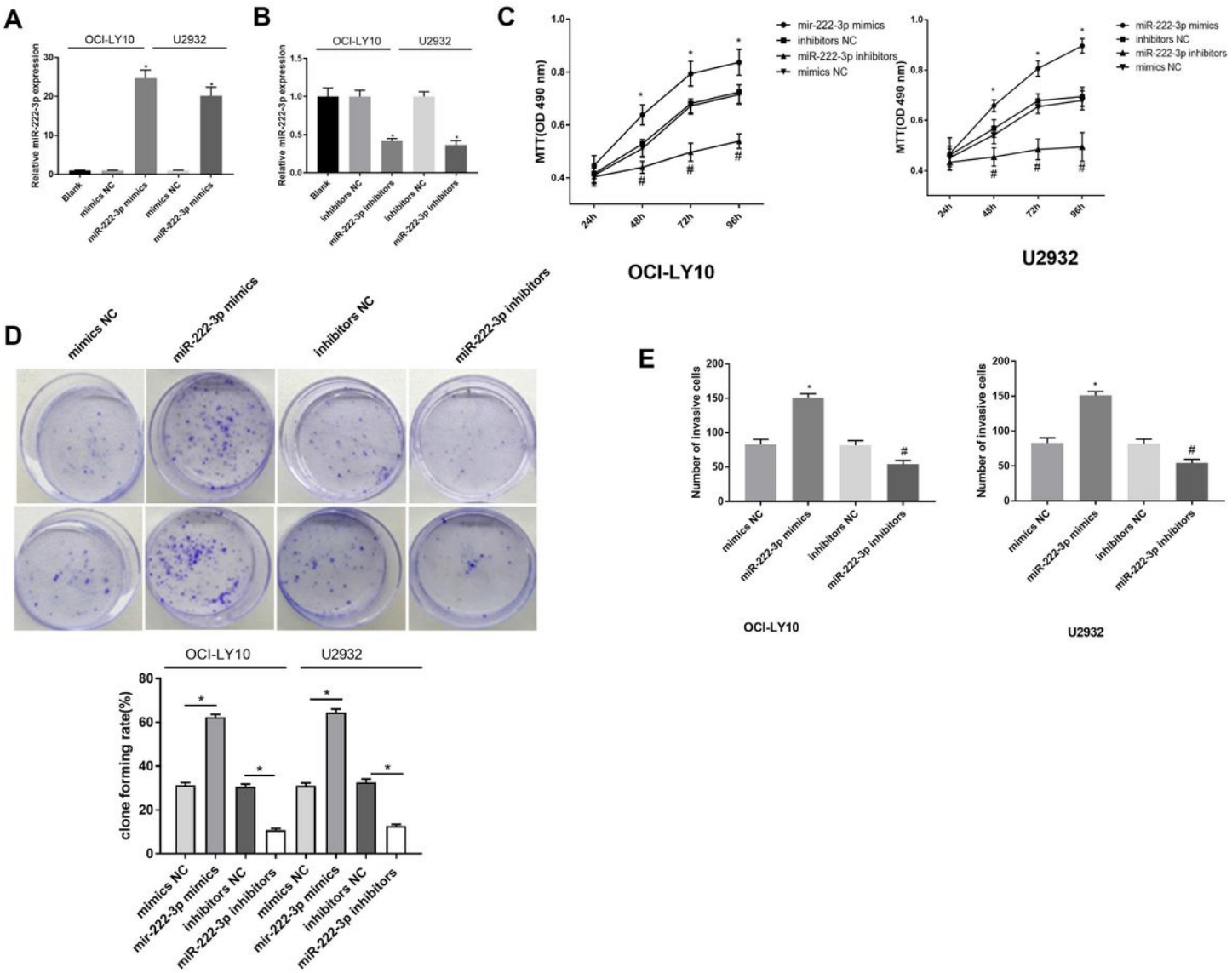

Figure 2

The effect of miR-222-3p in diffuse large B-cell lymphoma cell proliferation and invasion. A, qRT-PCR detection for miR-222-3p expression of miR-222-3p mimics or negative control transfected in OCI-LY10 and U2932 cells. B, qRT-PCR detection for miR-222-3p expression of miR-222-3p inhibitors or negative control transfected in OCI-LY10 and U2932 cells. C, proliferation of OCI-LY10 and U2932 cells detected by MTT assay. D, colony formation assay for cell proliferation. E, transwell invasion assay was used to detect the effects of microRNA-222-3p mimics or inhibitors on the invasion of OCI-LY10 and U2932 cells. *P < 0.05.

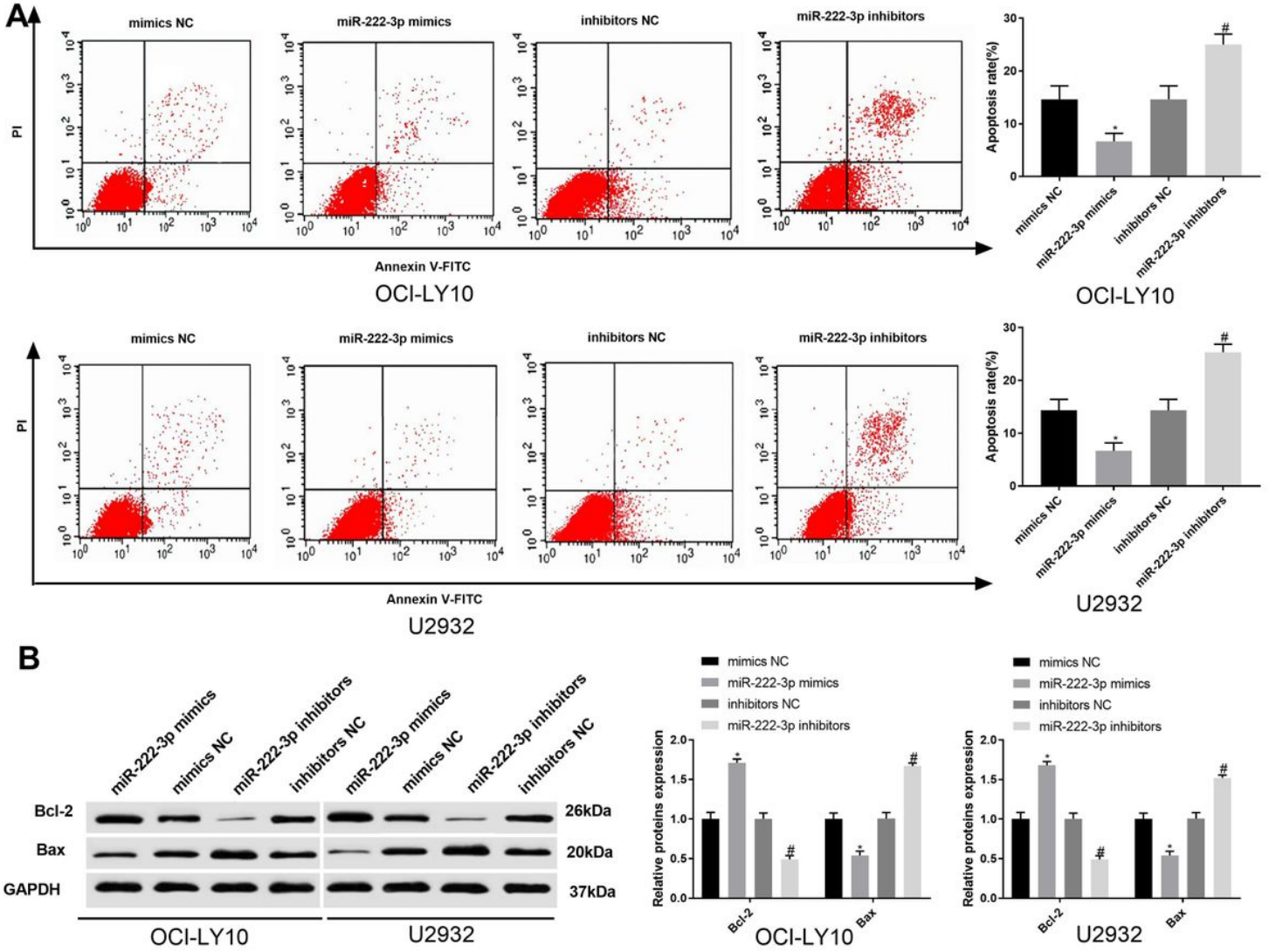

Figure 3

miR-222-3p inhibits apoptosis of diffuse large B-cell lymphoma cells. A, the effect of miR-222-3p mimics or inhibitors on apoptosis of OCI-LY10 and U2932 cells detected by flow cytometry. B, the effects of miR-222-3p mimics or inhibitors on the expression of Bcl-2 and Bax proteins in OCI-LY10 and U2932 cells detected by Western blot. Compared with Blank or mimics NC, *P < 0.05; compared with Blank or inhibitors NC, #P < 0.05. Data were expressed as mean ± standard deviation. All experiments were repeated 3 times.

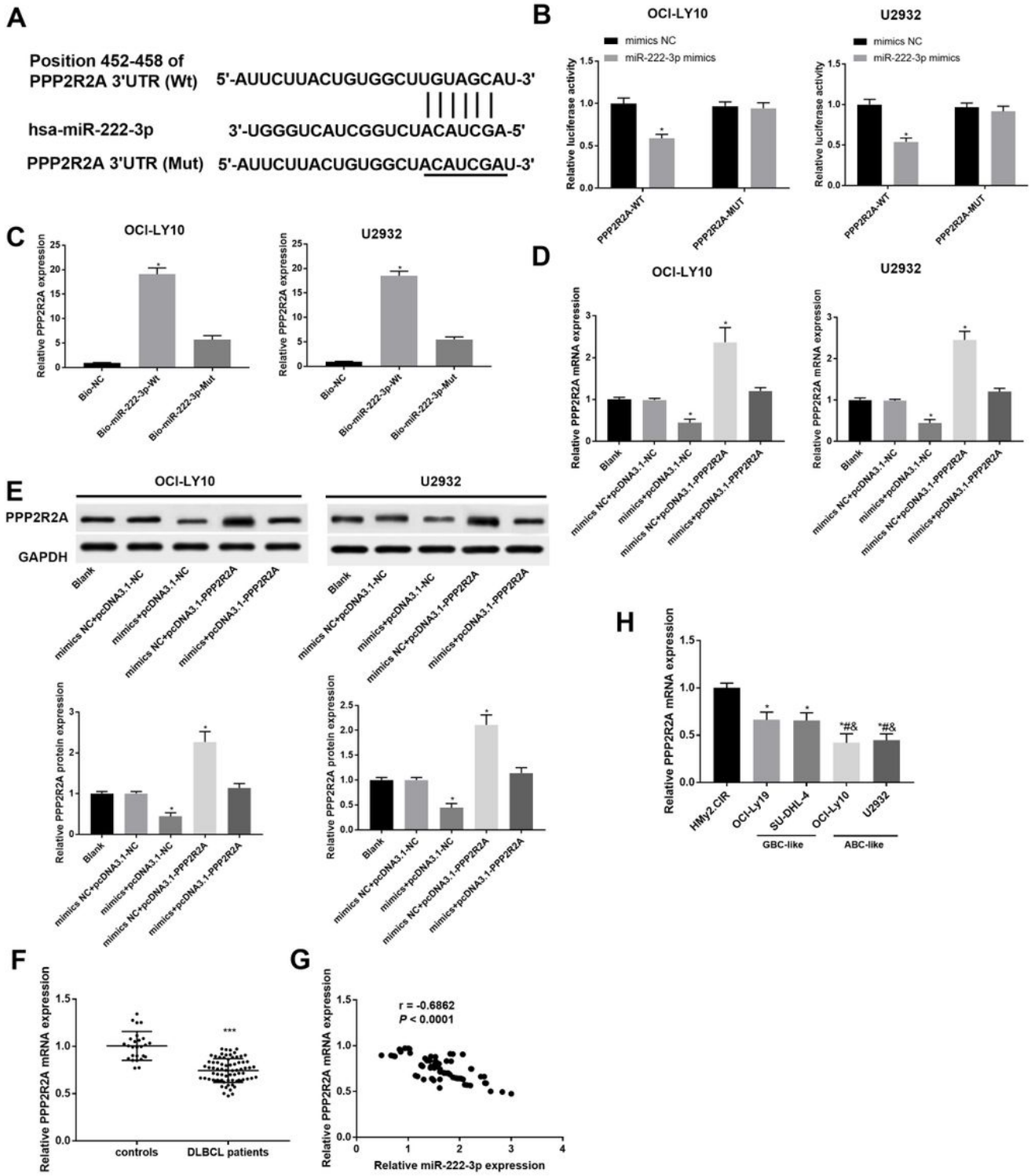

Figure 4

PPP2R2A is the target gene of miR-222-3p. A, the binding site of miR-222-3p to the 3'-UTR of PPP2R2A explored by Target Scan. B, dual luciferase reporter gene revealed that miR-222-3p binds to the 3' UTR sequence of PPP2R2A mRNA; compared with the co-transfection of miR-222-3p mimics and PPP2R2A-MUT, *P < 0.05. C, the binding ability between PPP2R2A and miR-222-3p in OCI-LY10 and U2932 cells confirmed by RNA pull down assay; compared with Bio-NC group, *P < 0.05. D, qRT-PCR was used to

detect PPP2R2A mRNA expression in OCI-LY10 and U2932 cells after transfection of miR-222-3p mimics or pcDNA3.1-PPP2R2A. E, Western blot was used to detect the expression of PPP2R2A protein in OCI-LY10 and U2932 cells transfected with miR-222-3p mimics or pcDNA3.1-PPP2R2A; compared with Blank or mimics NC+ pcDNA3.1-NC group, * $P < 0.05$. F, qRT-PCR was used to detect the expression level of PPP2R2A in patients with DLBCL and control group; compared with the control group, ***$P < 0.0001$. G, correlation analysis of miR-222-3p expression level and PPP2R2A expression level in DLBCL patients. H, qRT-PCR detection of DLBCL cell lines (OCI-LY19, SU-DHL-4, OCI-LY10 and U2932) and PPP2R2A expression levels in human normal B cell immortalized cell line (HMy2.CIR). Compared with HMy2.CIR, *$P <0.05$; compared with OCI-LY19, #$P<0.05$; compared with SU-DHL-4, & $P <0.05$. Data were expressed as mean ± standard deviation. All experiments were repeated 3 times.

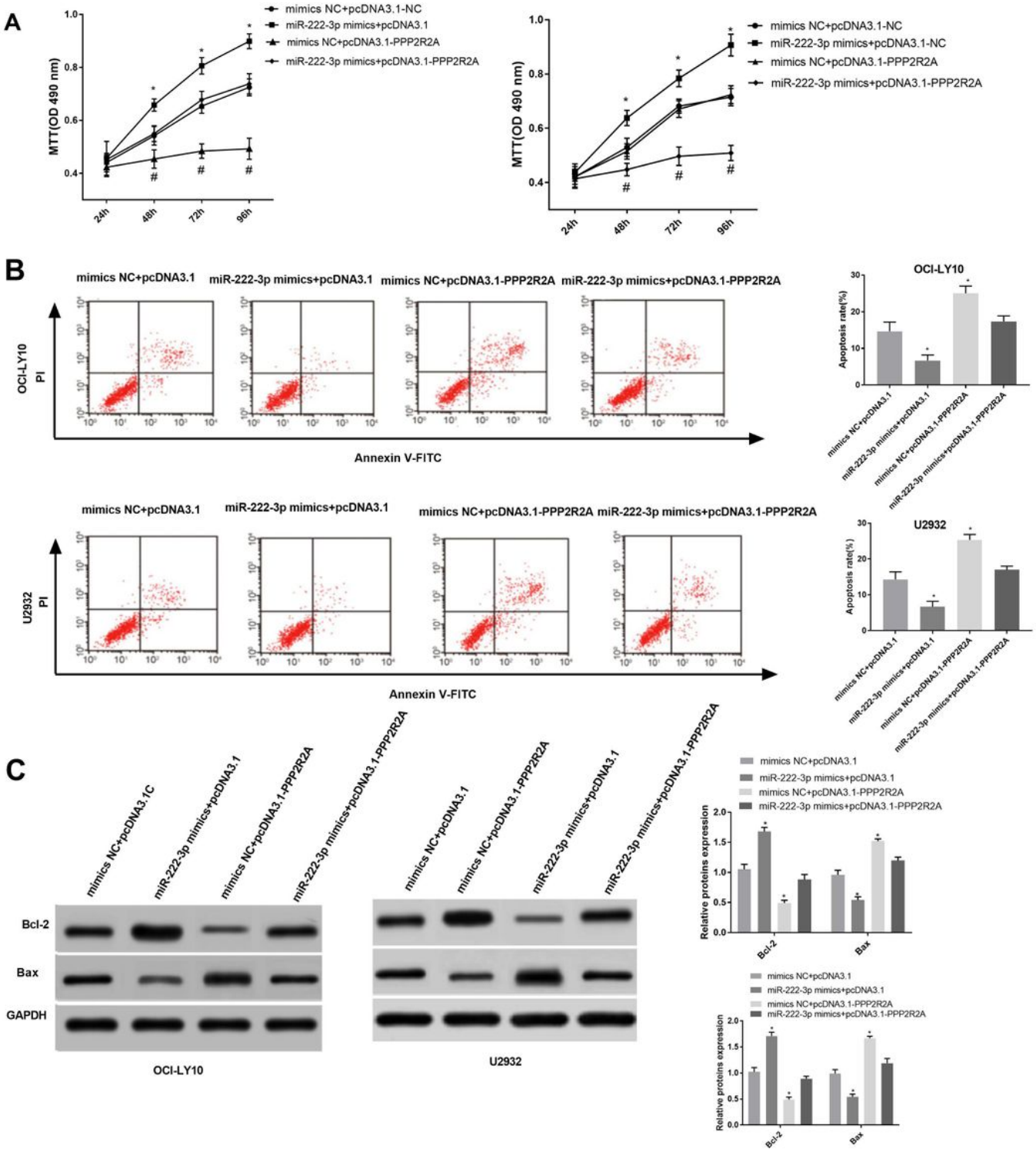

Figure 5

Overexpression of PPP2R2A regulated the effect of miR-222-3p on diffuse large B-cell lymphoma proliferation and apoptosis. A, the proliferation of miR-222-3p mimics or pcDNA3.1-PPP2R2A cells in OCI-LY10 and U2932 cells detected by MTT assay. B, the effect of miR-222-3p mimics or pcDNA3.1-PPP2R2A transfection on apoptosis of OCI-LY10 and U2932 cells detected by Flow cytometry. C, the effects of miR-222-3p mimics or pcDNA3.1-PPP2R2A transfection on the expression of Bcl-2 and Bax proteins in OCI-

LY10 and U2932 cells detected by Western blot. Compared to the Blank or mimics NC+ pcDNA3.1-NC group, *P < 0.05. Data were expressed as mean ± standard deviation. All experiments were repeated 3 times.

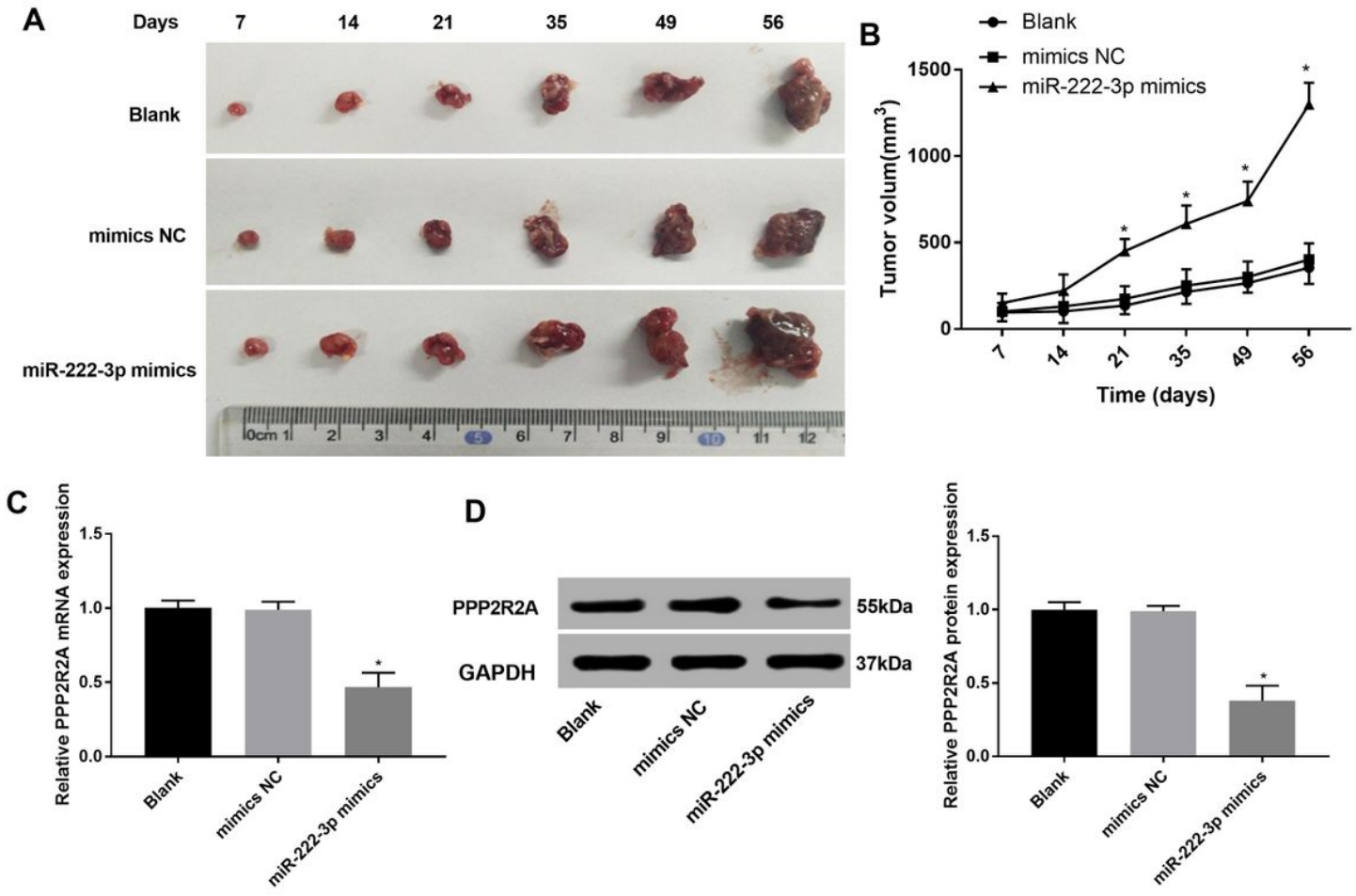

Figure 6

miR-222-3p mimics promotes tumor growth in nude mice. A, tumor image on the last day of the experiment (Day 56). B, miR-222-3p mimics promotes xenograft tumor growth in nude mice (tumor size is monitored every 7 days). C, detection of PPP2R2A mRNA expression in xenograft tumors by qRT-PCR. D, PPP2R2A expression in xenograft tumors detected by Western blot. Compared with the Blank or mimics NC group, *P < 0.05. Data were expressed as mean ± standard deviation. All experiments were repeated 3 times.

Supplementary Files

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