The regulation of miR-320a/XBP1 axis through LINC00963 for endoplasmic reticulum stress and autophagy in diffuse large B-cell lymphoma

Yuying Cui
Jiamusi University
Hui Xu
Jiamusi University
Yu Yang
Jiamusi University
Dongmei Zhao
Jiamusi University
Yu Wen
Jiamusi University
Chao Lv
Jiamusi University
Hongbin Qiu
Jiamusi University
Chennan Wang (Wchennan@jmsu.edu.cn)
Jiamusi University https://orcid.org/0000-0002-4783-6687

Research Article

Keywords: Bioinformatics, LINC00963, miR-320a, XBP1, ERs, apoptosis, autophagy

Posted Date: April 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-353877/v1

Abstract

Introduction: Huge amounts of gene-sequencing data have been used to guide fundamental researches. The study combined bioinformatics tools with basic study to analyze the pathological mechanisms of diffuse large B-cell lymphoma.

Methods: A LncRNA-miRNA-mRNA ceRNA network of diffuse large B cell lymphoma was constructed by GTEx combined with TCGA database analysis. qPCR was used to detect the expression of LINC00963 and miR-320a in DLBCL cell lines. The proteins levels of UPR sensors, GRP78, p-IRE1α, IRE1α, active ATF6, ATF4 and XBP1, were assessed through Western blot, along with apoptosis markers (Bcl-2, Bax, caspase 3) and autophagy indicators (Beclin1, LC3II, LC3I and p62) after LINC00963 overexpression or miR-320a overexpression in vitro. Additionally, the expression of LC3 was analyzed through immunofluorescence (IF) assay.

Results: Evaluation of SUDHL4 cell showed marked up-regulation of key elements of the UPR (GRP78, p-IRE1α, spliced XBP-1(XBP-1(s))), apoptosis (Bax, cleaved caspase 3) and autophagy (Beclin1, LC3II) after LINC00963 overexpression in vitro, whereas miR-320a mimic reversed the effects. Besides, LINC00963 targeted miR-320a while miR-320a bound to the 3'UTR of XBP1. The work also found that LINC00963 overexpression resulted in significant tumor growth delay in a xenograft model of DLBCL.

Conclusion: Mechanistically, LINC00963 / miR-320a regulated XBP1-apoptosis pathway and autophagy, making this pathway an attractive therapeutic target for selective targeting. The data presented here are the first to comprehensively survey the mechanism of LINC00963 / miR-320a/XBP1 in DLBCL.

Introduction

Diffuse large B-cell lymphoma (DLBCL), the most common form of non-Hodgkin's lymphoma (NHL), can account for 30–40%, is often progressive, and survival in untreated patients may be less than a year[1]. Understanding and studying the genesis and development mechanism of DLBCL could help to find its potential therapeutic targets and design new therapeutic schemes. Recent studies have shown that LncRNA account for about 80% of the total ncRNA and are involved in various physiological and pathological processes through transcriptional regulation, mRNA processing, spongy action, and nuclear transport[2, 3].

Many studies demonstrated that LINC00963 as a sponge of miRNA was involved in tumor growth[4–6]. miR-320a is located on human chromosome 8p21.3 and closely related to disease progression, tumor invasion and metastasis[7–10], the expression of which could be regulated by different lncRNA in different disease[8, 11, 12]. The endoplasmic reticulum (ER) stress or autophage-related proteins are correlated with worse overall survival of DLBCL patients and affected response of DLBCL to chemotherapy[13, 14]. In multiple myeloma, activation of the IRE1-XBP1 signaling pathway is thought to have a pro-oncogenic effect. However, in diffuse large B-cell lymphoma (GCB-DLBCL) derived from the germinal center, this pathway has a negative effect on tumor growth, so it is down-regulated. IRE1-XBP1

pathway plays a key role in malignant tumors, which can promote tumor growth or inhibit tumor growth, depending on the type of tumor. XBP1s may be directly involved in pro-apoptotic processes, which may contribute to the reduction of tumor growth in the lymphoma subtype[15].

Studies have shown that LINC00963 is up-regulated in some cancers, such as breast cancer and liver cancer, and plays a role in promoting cancer growth[16, 17]. However, we used GTEx database combined with TCGA database analysis to find that LINC00963 was down-regulated in GCB-DLBCL, and its low expression was significantly associated with poor prognosis. However, its role in diffuse large B-cell lymphoma needs further experiments.

Method

Database analysis

UCSC was used to download the data from GTEx and TCGA databases (normal N=337, tumor T=48), the intersection of genes was taken and corrected, and Wilcoxon test (non-parametric test) was used for difference analysis (logFC=1). The log-rank test in kaplan-Meier test was used to analyze the correlation of lncRNA expression and overall survival rate. Then, we used starbase 3.0 database to search for LncRNA that could bind to miRNA. The differentially expressed mRNAs in liver cancer were analyzed using non-parametric test (logFC=2) through Database of Essential Genes (DEG). The lncRNAs predicted above were used to predict their targeting miRNA through starbase 3.0 database. Then, we performed the prediction of mRNA using these these acquired miRNAs through miRDB, miRTarBase and TargetScan database, respectively. All three databases predicted mRNAs and differentially expressed mRNA from DEG were intersected. Cytoscape3.7.2 was used to construct the ceRNA network of LncRNA-miRNA-mRNA. At last, we performed GO enrichment analysis to evaluate the potential functions of RNA molecules in coexpression networks.

Cell culture

GM12878, SUDHL4, OCI-Ly1, HBL1 and OCI-Ly3 cells were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). GM12878 cells were cultured in RPMI 1640 medium (HyClone, USA) containing GlutaMAX Supplement (Thermo Fisher Scientific, USA, 15% fetal bovine serum (FBS, Sigma-Aldrich, USA) and 1% penicillin/streptomycin (Invitrogen, USA). SUDHL4 and OCI-Ly1were grown in DMEM medium with 10% FBS and 1% penicillin/streptomycin. HBL1 and OCI-Ly3 were cultured in Iscove's modified Dulbecco's medium (SIGMA, America) with 20% human serum medium and 1% penicillin/streptomycin.

qPCR

Total RNA of cells was extracted by Trizol method. The cells were centrifuged at 48,000 r/min for 5 min, the supernatant was discarded. 1 ml Trizol reagent was added. Then, the cells were placed for 15 min, mixed with 200μl chloroform. After 15min, the cells were centrifuged at 12000r/min at 4℃ for 10min.

The RNA was reverse transcribed into cDNA through cDNA reverse transcription kit according to manufacturer' instructions (TAKARA, Japan). The cDNA was amplified using SYBR PremixEx Taq kit (TAKARA, Japan). GADPH was used to be as internal reference. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of LINC00963, miR-320a, XBP1(s) mRNA and XBP1(u) mRNA.

Plasmids transfection

Plasmids overexpressing LINC00963 and the empty vector were purchased by Genechem (Shanghai, China). We purchased miR-320a mimic/NC inhibitor and miR-320a inhibitor/NC which were designed by GenePharma (Shanghai, China). The Lipotransfectamine 3000 was used to transfect SUDHL4 cells (Thermo Fisher Scientific, USA). After transfection of 48h, cells were used to perform next experiment.

CCK8

SUDHL4 cells were seeded into 96-well plate at a concentration of 100 μL/Well ($5*10^4$ cells) at 24, 48 and 72 h after transfection. 10 μL CCK-8 reagent was added to each well and incubated for 4 h. The absorbance (OD) at 450 nm was determined by a microplate microscope for 3 times. Each group was set with 5 multiple holes.

Western blot

After cells were treated, cells were collected, lysed on ice with RIPA lysate for 30 min and centrifuged at 12,000 g for 20 min to abstain supernatant. The cell lysis underwent SDS-PAGE electrophoresis and then transferred into PVDF membrane. Afterwards, protein bands were blocked with non-fat dry milk dissolved into PBS (5% wt/vol) for 1h and incubated with primary antibodies against GRP78, p-IRE1α,IRE1α, active ATF6, ATF4 XBP1(s), XBP1(u), Bcl-2, Bax, caspase 3, Beclin1, LC3II, LC3I and p62 (Abcam, England), respectively a 4 °C overnight. Then, protein bands were incubated with HRP-conjugated secondary antibodies (Abcam, England) for 2h at room temperature. The gray value was semi-quantified via Image J software.

Luciferase reporter assay

The pmirGLO dual-luciferase vector containing LINC00963 or XBP1 sequence (LINC00963 WT, XBP1 WT, respectively), or the pmirGLO dual-luciferase vector containing mutant LINC00963 or XBP1 sequence (LINC00963 MUT, XBP1 MUT) were co-transfected into SUDHL4 cells with miR-320a mimic or miR-320a. The fluorescence intensity was detected by Luciferase Reporter Assay System (Promega, USA).

Flow cytometry

Cells were collected after transfection of 48 h. $1*10^4$ cells were resuspended in 195μl Annexin V-FITC binding solution and 5μl Annexin V- FITC was added (Beyotime, Nanjing, China). Then, 10μl propidium iodide staining solution was added. After 20min, the apoptotic cells were detected through flow cytometry.

Immunofluorescent (IF) staining

SUDHL4 cells were fixed using 4% paraformaldehyde at 4 °C for 15 min after plasmids transfection, followed by permeabilized treatment with 0.2% Triton X-100 at room temperature for 15 min. Primary antibody against LC3 was added to incubate with cells at 4 °C overnight. Subsequently, cells were incubated with secondary antibodies for 90min. DAPI was used to stain nuclear. The cells were observed under an inverted microscope (Olympus, Tokyo, Japan).

Nude mouse xenograft model

SUDHL4 cells were cultured to logarithmic growth stage in vitro, digested by trypsin, washed using PBS and resuspended in RPMI 1640 medium. The cells ($2.5Í10^7$ cells/ml) were subcutaneously injected into the left back of nude mice. The size of the tumor was measured after subcutaneous tumor formation. The study was approved by the ethics committee of Jiamusi University.

Immunohistochemical assay

Paraffin sections were routinely dewaxed and underwent antigen retrieval under high pressure. The sections were then incubated with primary antibody against Ki-67 at 4℃ overnight (CST, USA). After being washed for three times using PBS. The second antibody was used to incubate with sections at room temperature for 20min. Then, the sections were stained with streptavidin-perosidase for 20min. Following being washed using PBS for three times, DAB was added to develop color. At last, the sections were observed under a microscope.

Statistical analysis

Experimental data were shown as mean±SD and analyzed using one-way analysis of variance followed by turkey's test. The statistical analysis was performed using Prism software. $P < 0.05$ as considered statistically significant.

Results

A diffuse large B cell lymphoma lncRNA-miRNA-mRNA ceRNA network was constructed by GTEx combined with TCGA database analysis

We searched USCS database for lncRNAs differently expressed in in DLBCL and performed heap map analysis as shown in Fig 1A. The log-rank test in kaplan-Meier test was used for the correlation analysis lncRNA expression and overall survival (Fig 1B), and 9 significantly correlated lncRNAs were obtained ($P < 0.05$). Then, we screened 5 LncRNAs which could bind to miRNA, including NRAV, LINC00937, LINC00963, DTX2P1-UPK3BP1-PMS2P1 and SNHG3, the expression levels of which in normal tissue and DLBCL were shown in Figure1C. Next step, the mRNAs differently expressed in DLBCL from DEG database were performed for the heap map analysis with Wilcoxon test (Fig1D). Based on co-expressed RNA and visualized via Cytoscape, we found that there was a targeting correlation among LINC00963/has-miR-

320a/XBP1 (Fig1E). GO analysis results revealed the involvement of twelve functional clusters including autophagy and the regulation of cell cycle (Fig1F), suggesting that the regulation function of these co-expressions of LncRNA in these processes.

Overexpression of LINC00963 inhibited proliferation of diffuse large B cell lymphoma cells

The expression of LINC00963 was detected in DLBCL and Human B lymphocytes (GM12878) through qPCR. We observed a marked low expression of LINC00963 in SUDHL4 cells as compared to GM12878 (Figure2A). Furthermore, LINC00963 expression obviously was lower than other DLBCL cells. Therefore, we predicted that LINC00963 could play a vital role in DLBCL. Next, DLBCL cell overexpressing LINC00963 was constructed which was confirmed through qPCR (Figure2B). we further found that LINC00963 overexpression significantly inhibited cell proliferation in time-dependent manner (Figure2C).

Overexpression of LINC00963 affects endoplasmic reticulum stress, apoptosis and autophagy

The induction of exogenous LINC00963 upregulated the protein levels of GRP78 and phosphorylated IRE1α, but not significantly affect the protein levels of IRE1α, ATF6 and ATF4 (Figure3A). Moreover, the ratio of XBP1(s) and XBP1(u) was markedly elevated. It can be seen that LINC00963 overexpression mainly activates IRE1/XBP pathway. Excess ER stress could induce cell apoptosis. Therefore, we further analyze the effect of LINC00963 overexpression on SUDHL4 cells. As shown in Figure 3B, LINC00963 overexpression group showed more apoptotic cells through Flow cytometry analysis, along with increased protein levels of Bax and cleaved caspase3 as well as reduction in Bcl-2 expression (Figure3C). Previously, the results of Go function enrichment implied that co-expression of LncRNAs was involved in regulating autophagy in DLBCL. To further investigate the function of LNC00963, the expression of LC3 was detected through immunofluorescence assay. There was a significant increase in green LC3 dots in Oe-LINC00963 group relative to Oe-NC group (Figure3D). Western blots further revealed that LNC00963 overexpression promoted the expression of Beclin1 and LC3II while inhibited p62 expression (Figure3E). Furthermore, the protein levels of LC3I didn't exhibit significant changes after LNC00963 overexpression (Figure3E). Based on these results, LINC00963 overexpression induces autophagy in SUDHL4 cells.

miR-320a is the direct target of LINC00963

ceRNA network of lncRNA-miRNA-mRNA has previously revealed that a targeting regulatory relationship between LINC00963 and miR-320a possibly exists in DLBCL. So miR-320a expression was further determined and was found to show relatively higher levels in DLBCL cell lines versus GM12878 cells (Figure4A). Bioinformatics analysis showed that LNC00963 contained a highly conserved miR-320a binding site as shown in Figure 4B, and dual luciferase analysis and RT-PCR were used to further determine the predicted target. Luciferase activity was significantly decreased in the LINC00963 WT (wild type) group, while there was no significant change in the LINC00963 MUT (mutant type) group (Figure 4C). We further found that LINC00963 overexpression significantly reduced the expression of miR-320a (Fig 4D), suggesting that LINC00963 may have a target-specific relationship with miR-320a.

XBP1 is the target protein of miR-320a in SUDHL4 cells

The results of bioinformatics analysis indicated that LINC00963/ miR-320a could regulate the proteins levels of XBP1. Next, we further analyze the expression of XBP1 in GM12878 and DLBCL cells. There was a significant reduce in the ratio of XBP1s/u in SUDHL4 than that in GM12878 cells. Surprisingly, the ratio of XBP1s/u seemed to lower than other DLBCL cells (Fig5A). Bioinformatics studies showed that miR-320a had complementary binding sites with 3'-UTR of XBP1 mRNA (Fig5B). XBP1 protein has two kinds of form, unspliced XBP1u and spiced XBPls. When cells underwent ERs, the endonuclease activity of IRE1 dissociating from sugar-regulating protein GRP78 was activated through self-dimerization and phosphorylation. Then, IRE1 specifically removes an intron fragment of the sequence of 26bp in length and finally leads to the production of XBP1s which was involved in the expansion of endoplasmic reticulum, protein maturation, misfolded protein degradation and other processes[18, 19].

We co-transfected SUDHL4 cells with XBP1 3’ UTR-WT/Mut and miR-320a for luciferase detection. As shown in Fig 5C-D, XBP1 mRNA 3'-UTR has a targeted relationship with miR-320a. Next, we further determined the functional relationship between XBP1 and miR-320a. Cells were transfected with miR-320a inhibitor and miR-320a-NC, respectively. The results showed that the ratio of XBP1 s/u expression showed obvious increase in cells transfected with miR-320a inhibitor (Fig 5E).

miR-320a mimic reversed the proliferation, endoplasmic reticulum stress, apoptosis and autophagy induced by LINC00963 overexpression

The following experiments were designed to investigate whether LNC00963 regulates the viability of SUDHL4 cells of by regulating miR-320a. The cell proliferation rate of Oe-LINC00963 group was lower than that of Oe-NC group, while the results were opposite in Oe-LINC00963 + miR-320a mimic group (Fig6A). In addition, protein expression levels of GRP78, p-IRE1α IRE1α, active ATF6, ATF4, XBP1(s) and XBP1(u) were measured in different transfection groups (Fig6B). The results showed that protein expression levels of GRP78, p-IRE1α and XBP1(s) were significantly increased in Oe-LINC00963 group, while the results were opposite in Oe-LINC00963 + miR-320a mimic group (Fig6B). Next step, we further analyzed how LINC00963 affected cell apoptosis. We conducted flow cytometry assay, and the results showed that cell apoptosis was significantly increased after transfection of Oe-LINC00963, while miR-320a mimic could counteract the effect of LINC00963 overexpression on SUDHL4 cells apoptosis after co-transfection of Oe-LINC00963 and miR-320a (Figure6C). As shown in Figure 6D, after LINC00963 overexpression, the expression of key factors in apoptotic pathway was changed (decreased Bcl-2 expression, and increased Bax and cleaved caspase3). However, the effects of LINC00963 overexpression were counteracted after Oe-LINC00963 and miR-320a mimic were transfected into SUDHL4 cells (Fig6D). It was proved that down-regulation of LINC00963 could activate the apoptosis signaling pathway of SUDHL4 cells and induce apoptosis by regulating miR-320a.

Next, how LINC00963 regulated autophagy was further investigated. Immunofluorescence assay implied that miR-320a mimic dulled the effects of LINC00963 overexpression on promoting the expression of LC3II and the inhibitory effects on the expression of LC3I (Fig6E). Beclin1 and LC3II protein expressions

were increased and p62 expression were elevated in Oe-LINC00963 group compared with the NC group, the effects of which were blocked after the co-transfection of Oe-LINC00963 and miR-320a mimic into SUDHL4 cells (Fig6F).

Overexpression of LINC00963 inhibits the growth of diffuse large B cell lymphoma in vivo

Tumorigenesis was performed in nude mice by subcutaneous injection of LINC00963 overexpressing cells. Tumor formation in nude mice was effectively inhibited (Fig7A), and tumor volume (Fig7B) and weight (Fig7C) were significantly reduced. LINC00963 overexpression effectively inhibited tumor growth, which was consistent with the results observed in cell experiments of LIN00473 overexpression. Ki67, as a tumor cell proliferation marker, was found to present higher expression levels LIN00473 in overexpression group as compared to control group (Figure7D). Next, the changes of LINC00963, miR-320a, XBP1(s) and XBP1(u) were detected, respectively by qPCR. The results showed that in the LINC00473 overexpression group, the expression of LINC00963 and XBP(s) was elevated while the expression of miR-320a and XBP1(u) was decreased (Fig7E).

Discussion

LINC00963 has been reported to play oncogenic roles in several cancer types including osteosarcoma and breast cancer[16, 20]. In the present study, the expression of LINC00963 showed significant lower levels in DLBCL tissue compared with normal tissue through analysis for TCGA data, which was further confirmed by in vitro and in vivo experiment. Mechanistically, lncRNA-miRNA-mRNA ceRNA network revealed that there was a target correlation among LINC00963, miR-320a and XBP1, suggesting that LINC00963/miR-320a/XBP1 could implicate in regulating cell biological process. The results of in vitro and in vivo experiment revealed that LINC00963/ miR-320a axis regulated ER stress, apoptosis and autophagy process.

Targeting protein degradation pathways was considered as a novel approach for the inhibition of lymphoma growth[21]. LINC00963 overexpression contributed to induction of UPR with increased ratio of XBP1(s)/XBP1(u), the phosphorylation level of IRE1α and GRP78 protein levels, but no change occurred in ATF6 and ATF4, indicating that LINC00963 promoted UPR mainly through regulating IRE1/XBP pathway. Then, the result of flow cytometry showed the promotion of SUDHL4 cell apoptosis after LINC00963 overexpression, along with a reduction in Bcl-2 expression and an increase in the protein levels of Bax and cleaved caspase 3, further indicated that LINC00963 activated UPR-cell apoptosis possibly through regulating IRE1/XBP pathway. It has been recognized that there is a pro-apoptosis effect in the activation of GRP-78/IRE-1/XBP-1 signaling pathway[22, 23].

These lncRNA molecules closely associated with the prognostic of DLBCL patients, were found to be enrich in autophagy pathway through GO function enrichment analysis. The hypothesis that emerges is that LINC00963 could regulate autophagy pathway. Surprisingly, the detection of the expression of autophagy-related markers in vitro and vivo DLBCL models validated these findings. IRE1α-XBP1 pathway was also reported to partly be responsible to induction of autophagy in cancer[24, 25].

The oncogenic activity of LINC00963 has been confirmed in some cancer, such as osteosarcoma, breast cancer and ovarian cancer[6, 16, 26]. Base on the summary of the results of recent research, we found that LINC00963 not only affected tumorigenesis through functioning as a sponge of miRNA[27], but regulated the sensitivity of tumor cells to chemotherapy and radiotherapy[16, 28]. Conversely, our work demonstrated that LINC00963 overexpression facilitated DLBCL cell apoptosis in vitro and inhibited tumor progression in vivo, which provides a new sight to understand the function of LINC00963 in DLBCL. Luciferase reporter and qPCR assay revealed that LINC00963 sponged the 3'UTR of miR-320a to induce a reduce in the miR-320a expression. Additionally, in SUDHL4 cell, miR-320a overexpression rescued the effects of LINC00963 overexpression in UPR, cell apoptosis and autophagy. These findings demonstrated that LINC00963 could directly regulate miR-320a levels to affect aforementioned biological process of cells. Besides, miR-320a was validated to target XBP1 mRNA and its inhibitor elevated the ratio of XBP1(s)/XBP1(u). Taken together,

LINC00963 promoted XBP1-cell apoptosis and autophagy depending on miR-320a. In conclusion, this study also elucidated the regulatory pathway of LINC00963/miR-320a/XBP1 and its biological function in tumors, suggesting that both LINC00963 and miR-320a could be potential therapeutic targets, providing a new idea for molecular targeted therapy of DLBCL.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Jiamusi University.

Consent for publication

Not applicable.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

Funding

The study was supported by Scientific Research Fund of Heilongjiang Provincial Education Department (No.2018-KYYWF-0932) and North Medicine and Functional Food Characteristic Subject Project in Heilongjiang Province (No.2018-TSXK-02).

Authors' contributions

Yy C and Cn W made substantial contributions to the conception and design of the study, acquired, analyzed and interpreted the data, and drafted and revised the manuscript for important intellectual content; Yy C, H X, Y Y, Dm Z, YW, CL, Hb Q and CN W performed the experiments and interpreted the data. All authors read and approved the final manuscript.

Acknowledgements

No applicable.

References

1. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, Lih CJ, Williams PM, Shaffer AL, Gerecitano J, de Vos S, Goy A, Kenkre VP, Barr PM, Blum KA, Shustov A, Advani R, Fowler NH, Vose JM, Elstrom RL, Habermann TM, Barrientos JC, McGreivy J, Fardis M, Chang BY, Clow F, Munneke B, Moussa D, Beaupre DM, Staudt LM. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med. 2015;21:922–6.
2. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature. 2014;505:344–52.
3. Lv Z, Xu Q, Yuan Y. A systematic review and meta-analysis of the association between long non-coding RNA polymorphisms and cancer risk. Mutation Research/reviews in Mutation Research 2016; S1383574216301028.
4. Wu W, Wang W, Yao S. Zhang and Ren. Long noncoding RNA LINC00963 promotes breast cancer progression by functioning as a molecular sponge for microRNA-625 and thereby upregulating HMGA1. Cell Cycle 2020; 19.
5. Wei L, Yu-Jia Y, Qiang A. LINC00963 Promotes Ovarian Cancer Proliferation, Migration and EMT via the miR-378g / CHI3L1 Axis. Cancer management and research 2020; 12.
6. Feng S, Ke W, Zhixian Y, Xingyu M, Zhong Z, Menghao S, Yong W, Zhihong L, Yiyong Z. Long noncoding RNA LINC00963 induces NOP2 expression by sponging tumor suppressor miR-542-3p to promote metastasis in prostate cancer. Aging 2020; 12.
7. Hao X, Xin R, Dong W. Decreased serum exosomal miR-320a expression is an unfavorable prognostic factor in patients with hepatocellular carcinoma. Journal of International Medical Research 2020; 48.
8. Huang, Zhu, Ke X, Liang C, Chang. LncRNA FTX inhibition restrains osteosarcoma proliferation and migration via modulating miR-320a/TXNRD1. Cancer Biology & Therapy 2020; 21.
9. Prado MSG, Jesus MLd G, TCd, Mendonça LSO, Kaneto CM. Downregulation of circulating miR-320a and target gene prediction in patients with diabetic retinopathy. BMC Research Notes 2020; 13.
10. Zhang C, Yang H, Li Y, Huo P, Ma P. LNCRNA OIP5-AS1 regulates oxidative low-density lipoprotein-mediated endothelial cell injury via miR-320a/LOX1 axis. Molecular and Cellular Biochemistry: An International Journal for Chemical Biology in Health and Disease 2020; 467.

11. Rongrong Z, Xiao H, Wei X, Zhourui W, Yanjing Z, Yilong R, Liming C. LncRNA MALAT1 inhibits hypoxia/reoxygenation-induced human umbilical vein endothelial cell injury via targeting the microRNA-320a/RAC1 axis. Biological chemistry 2020; 401.
12. Wu S, Chen S, Lin N, Yang J. Long non-coding RNA SUMO1P3 promotes hepatocellular carcinoma progression through activating Wnt/β-catenin signalling pathway by targeting miR-320a. Journal of Cellular and Molecular Medicine 2020; 24.
13. Mozos A, Roué G, López-Guillermo A, Jares P, Campo E, Colomer D, Martinez A. The Expression of the Endoplasmic Reticulum Stress Sensor BiP/GRP78 Predicts Response to Chemotherapy and Determines the Efficacy of Proteasome Inhibitors in Diffuse Large B-Cell Lymphoma. The American Journal of Pathology 2011; 179.
14. Jia-Jia H, Ying-Jie Z, Tong-Yu L, Wen-Qi J, Hui-Qiang H, Zhi-Ming L. Beclin 1 expression predicts favorable clinical outcome in patients with diffuse large B-cell lymphoma treated with R-CHOP. Human pathology 2011; 42.
15. Bujisic B, De Gassart A, Tallant R, Demaria O, Zaffalon L, Chelbi S, Gilliet M, Bertoni F, Martinon F. Impairment of both IRE1 expression and XBP1 activation is a hallmark of GCB DLBCL and contributes to tumor growth. Blood. 2017;129:2420–8.
16. Zhang N, Zeng X, Sun C, Guo H, Wang T, Wei L, Zhang Y, Zhao J, Ma X. LncRNA LINC00963 Promotes Tumorigenesis and Radioresistance in Breast Cancer by Sponging miR-324-3p and Inducing ACK1 Expression. Mol Ther Nucleic Acids. 2019;18:871–81.
17. Wu JH, Tian XY, An QM, Guan XY, Hao CY. LINC00963 promotes hepatocellular carcinoma progression by activating PI3K/AKT pathway. Eur Rev Med Pharmacol Sci. 2018;22:1645–52.
18. Naidoo N. ER and aging-Protein folding and the ER stress response. Ageing Res Rev. 2009;8:150–9.
19. Fung TS, Liao Y, Liu DX. The endoplasmic reticulum stress sensor IRE1α protects cells from apoptosis induced by the coronavirus infectious bronchitis virus. J Virol. 2014;88:12752–64.
20. Zhou Y, Yin L, Li H, Liu LH, Xiao T. The LncRNA LINC00963 facilitates osteosarcoma proliferation and invasion by suppressing miR-204-3p/FN1 axis. Cancer Biol Ther. 2019;20:1141–8.
21. Amengual JE, Johannet P, Lombardo M, Zullo K, Hoehn D, Bhagat G, Scotto L, Jirau-Serrano X, Radeski D, Heinen J, Jiang H, Cremers S, Zhang Y, Jones S, O'Connor OA. Dual Targeting of Protein Degradation Pathways with the Selective HDAC6 Inhibitor ACY-1215 and Bortezomib Is Synergistic in Lymphoma. Clin Cancer Res. 2015;21:4663–75.
22. Banerjee A, Ahmed H, Yang P, Czinn SJ, Blanchard TG. Endoplasmic reticulum stress and IRE-1 signaling cause apoptosis in colon cancer cells in response to andrographolide treatment. Oncotarget. 2016;7:41432–44.
23. Iurlaro R, Muñoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. Febs j. 2016;283:2640–52.
24. Zhang X, Hu P, Ding SY, Sun T, Liu L, Han S, DeLeo AB, Sadagopan A, Guo W, Wang X. Induction of autophagy-dependent apoptosis in cancer cells through activation of ER stress: an uncovered anti-cancer mechanism by anti-alcoholism drug disulfiram. Am J Cancer Res. 2019;9:1266–81.

25. Thakur PC, Miller-Ocuin JL, Nguyen K, Matsuda R, Singhi AD, Zeh HJ, Bahary N. Inhibition of endoplasmic-reticulum-stress-mediated autophagy enhances the effectiveness of chemotherapeutics on pancreatic cancer. J Transl Med. 2018;16:190.
26. Liu W, Yang YJ, An Q. LINC00963 Promotes Ovarian Cancer Proliferation, Migration and EMT via the miR-378g /CHI3L1 Axis. Cancer Manag Res. 2020;12:463–73.
27. Wang J, Li C, Xu L, Yang C, Zhang X. MiR-1193 was sponged by LINC00963 and inhibited cutaneous squamous cell carcinoma progression by targeting SOX4. Pathol Res Pract. 2019;215:152600.
28. Lee SP, Hsieh PL, Fang CY, Chu PM, Liao YW, Yu CH, Yu CC, Tsai LL. LINC00963 Promotes Cancer Stemness, Metastasis, and Drug Resistance in Head and Neck Carcinomas via ABCB5 Regulation. Cancers (Basel) 2020; 12.

Figures

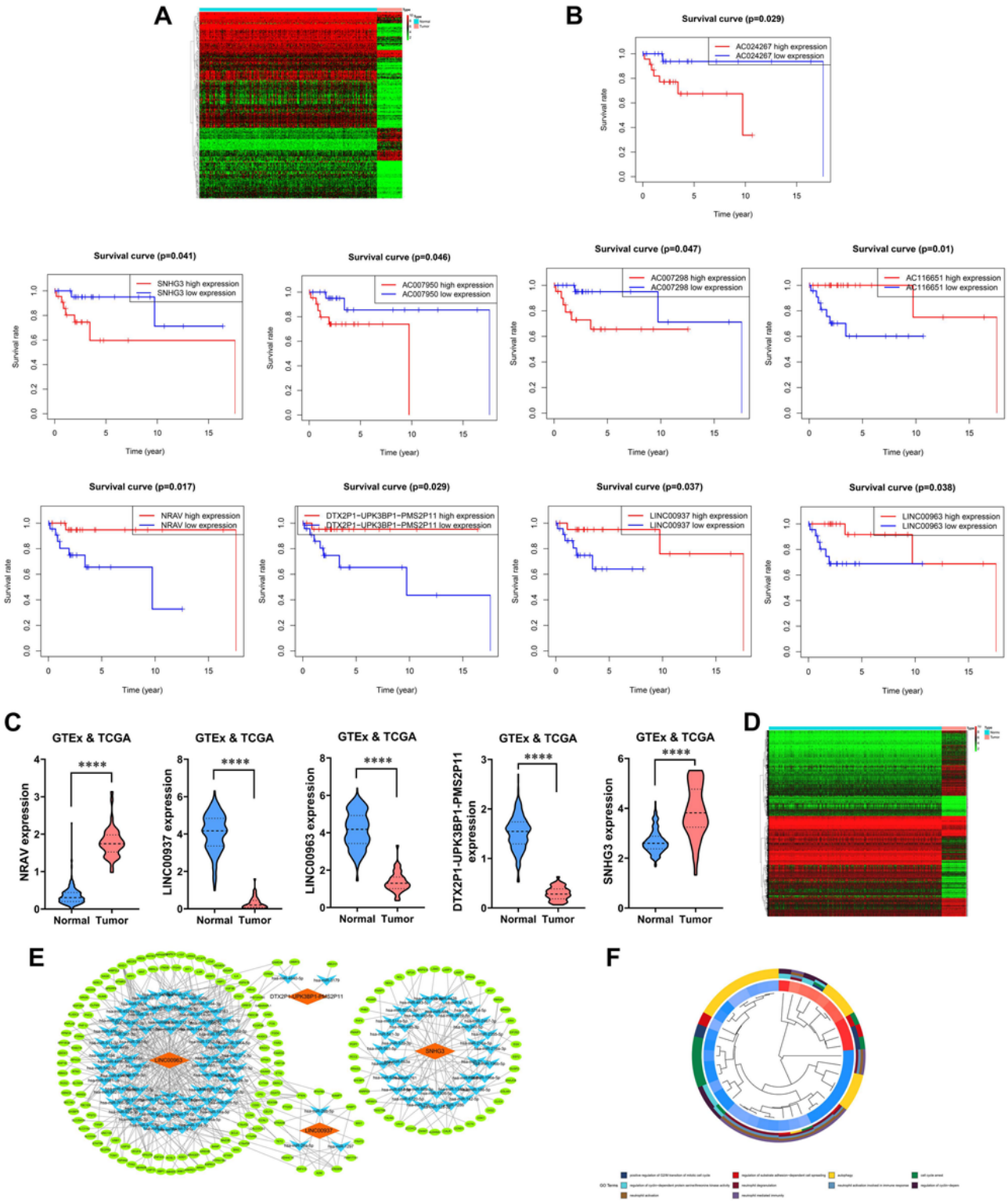

Figure 1

A the analysis of heap map for DLBCL data of UCSC database (normal N=337, DLBCL T=48). B The Kaplan–Meier survival analysis about LncRNA in DLBCL patients. C the expression of NRAV、LINC00937、LINC00963、DTX2P1-UPK3BP1-PMS2P11 and SNHG3 in DLBCL. D Heap map about mRNAs differently expressed in DLBCL. E ceRNA network of lncRNA-miRNA-mRNA. F Functional enrichment analysis of co-expressed genes.

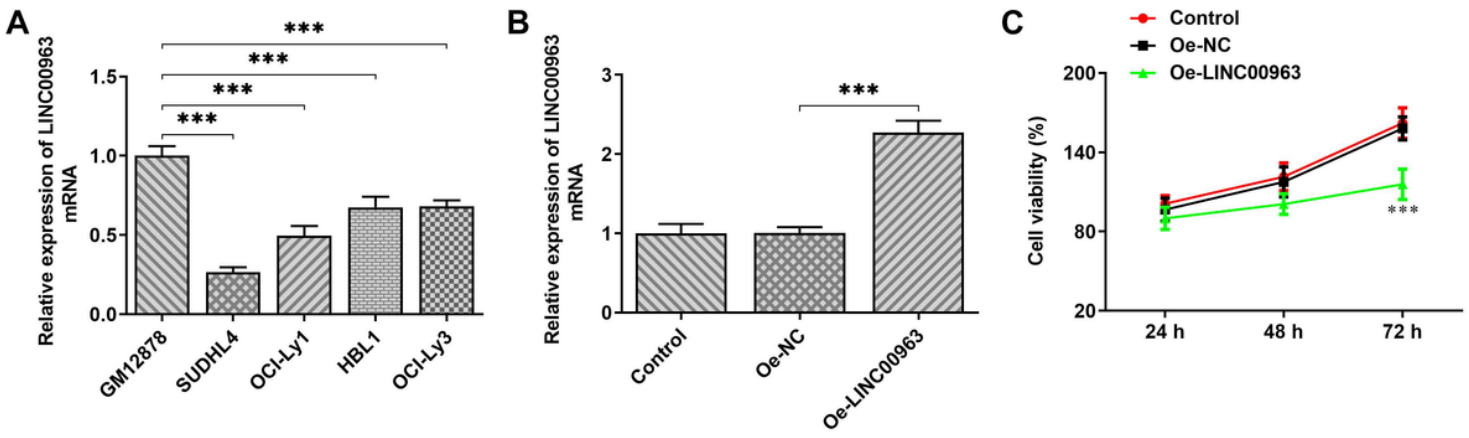

Figure 2

A The expression of LINC00963 in diffuse large B-C cell lymphoma was detected by qPCR. B LINC00963 overexpressed plasmid elevated LINC00963 levels and enhanced the proliferation of DLBCL cell.

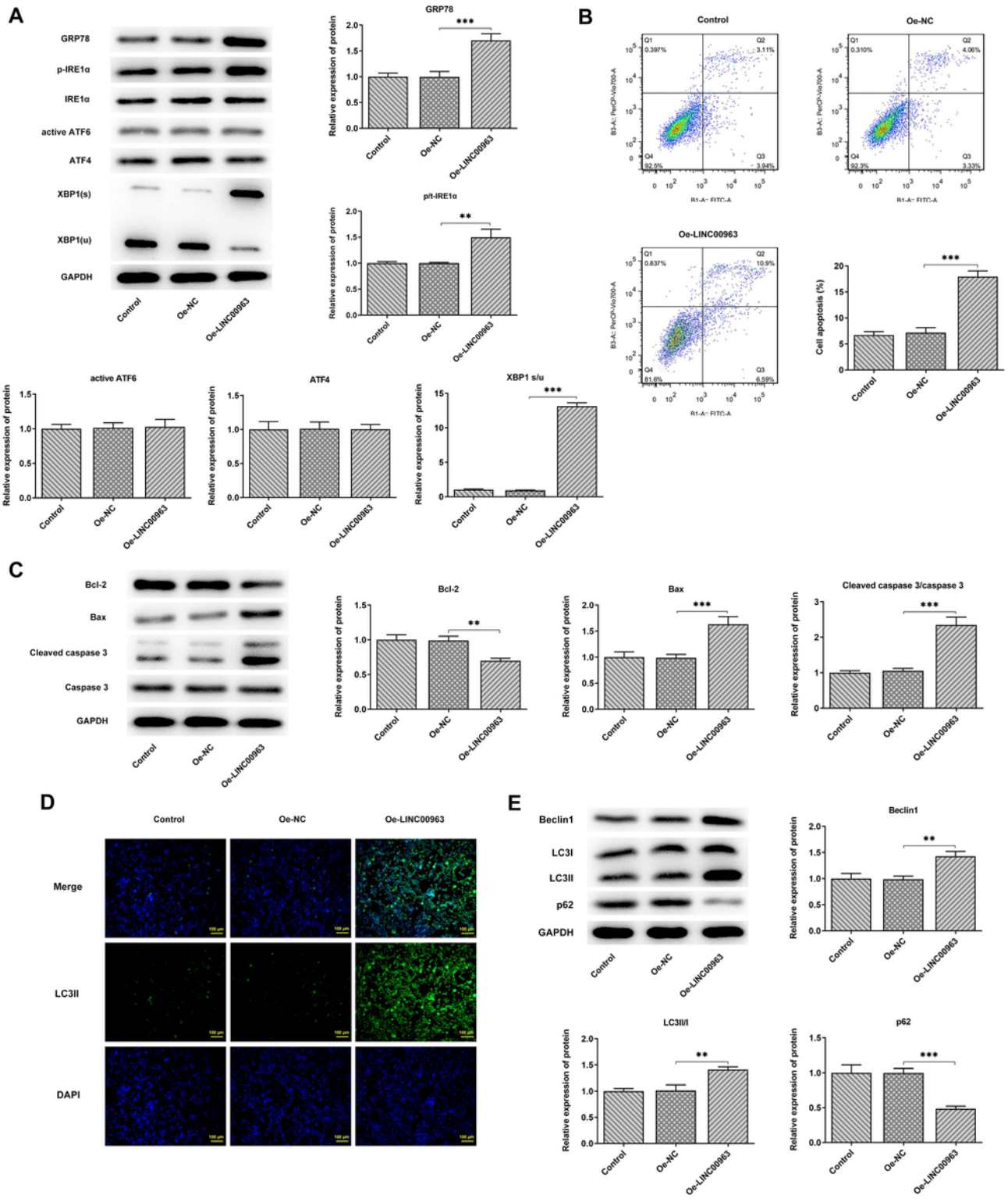

Figure 3

A DLBCL cells were analyzed for GRP78, p-IRE1α IRE1α, active ATF6, ATF4 XBP1(s) and XBP1(u) by Western blot. B Flow cytometry was used to detect DLBCL apoptosis after LINC00963 overexpression. C LINC00963 overexpression affected apoptosis-related proteins levels. D. Immunofluorescence analyzed the expression of LC3. E The detection of autophagy markers (Beclin1, LC3II, LC3I and p62) through WB.

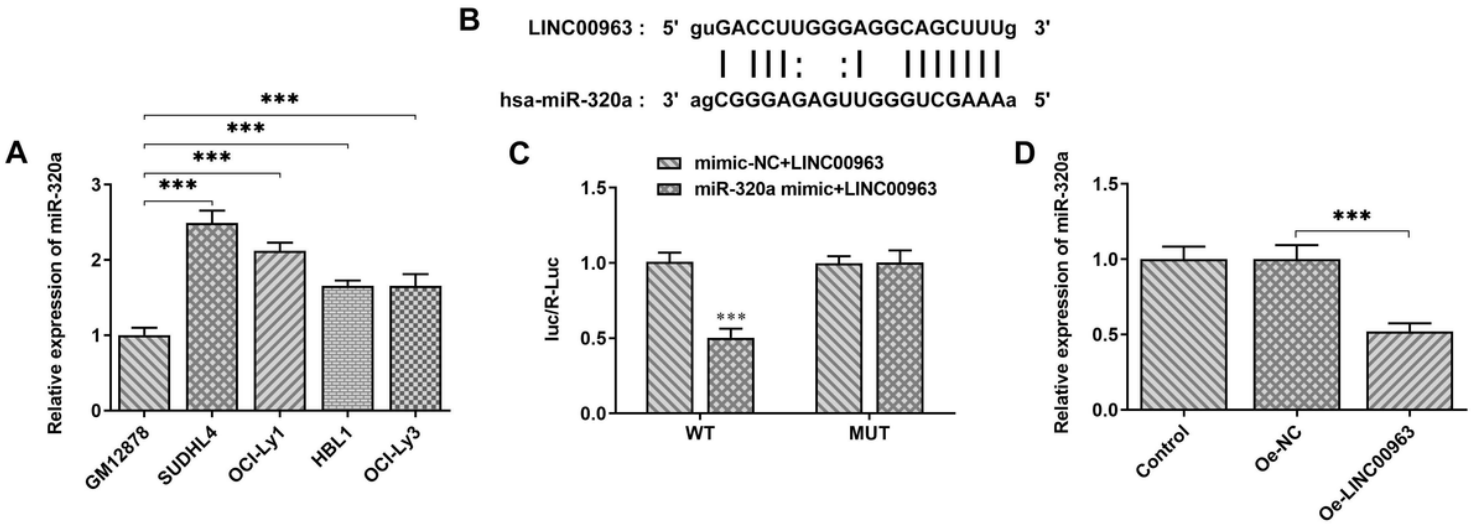

Figure 4

A The expression of miR-320a in diffuse large B cell lymphoma cell lines was detected by qPCR. B Starbase 3.0 predicted the binding sites of LINC00963 and miR-320a. C Luciferase activity was detected. D LINC00963 overexpression reduced the expression of miR-320a.

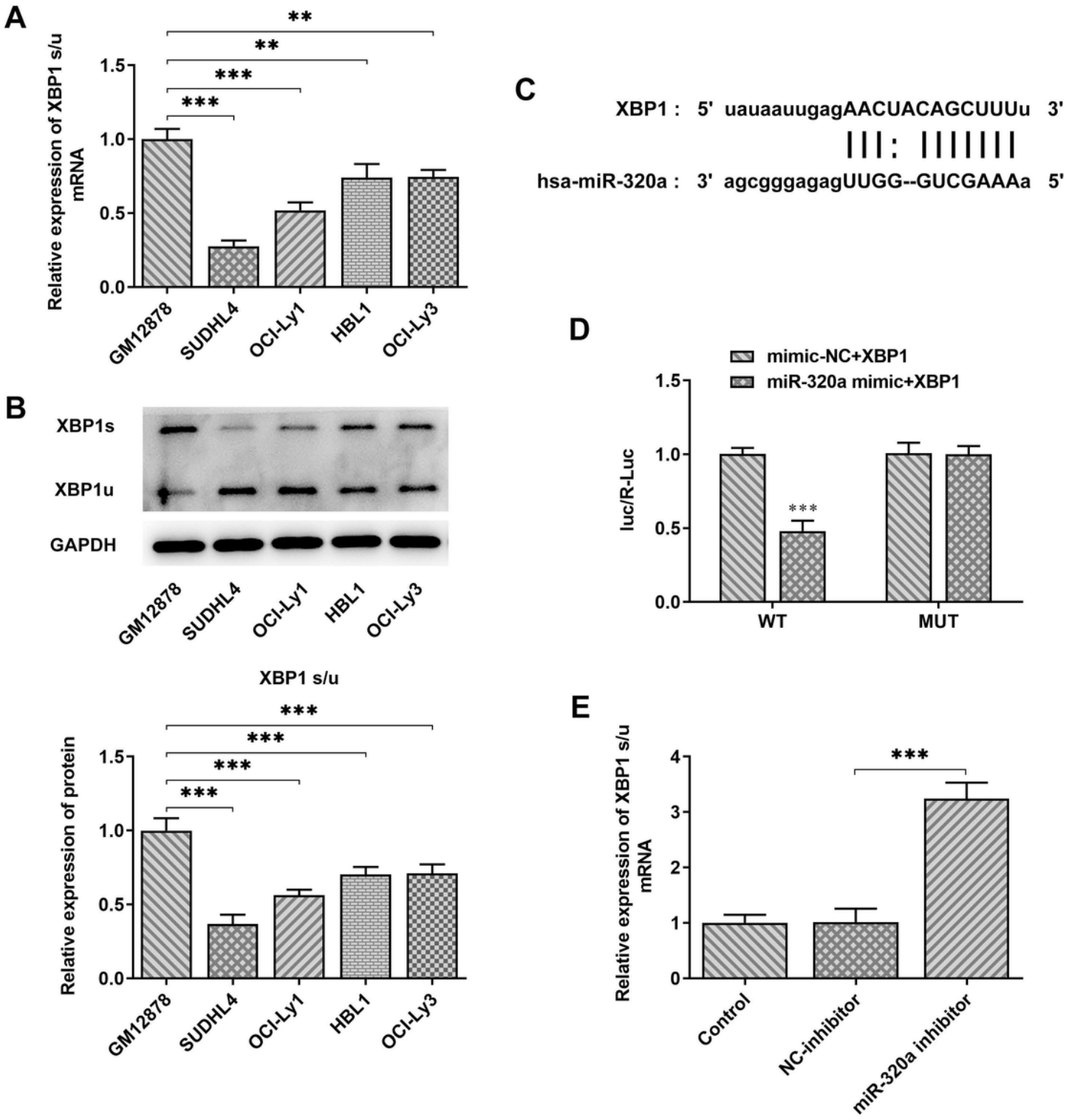

Figure 5

A The expression of XBP1 in diffuse large B cell lymphoma cell lines was detected by qPCR and Western blot, and the ratio of XBP1s/u was calculated. B miR-320a has binding sites with XBP1. C Luciferase activity was detected by double luciferase assay after miR-320a mimic was co-transfected with wild or mutant XBP1 3' UTR. D XBP1(u) and XBP1(s) mRNA expression was detected by RT-QPCR, respectively, after transfection of Mir-361-5p inhibitor.

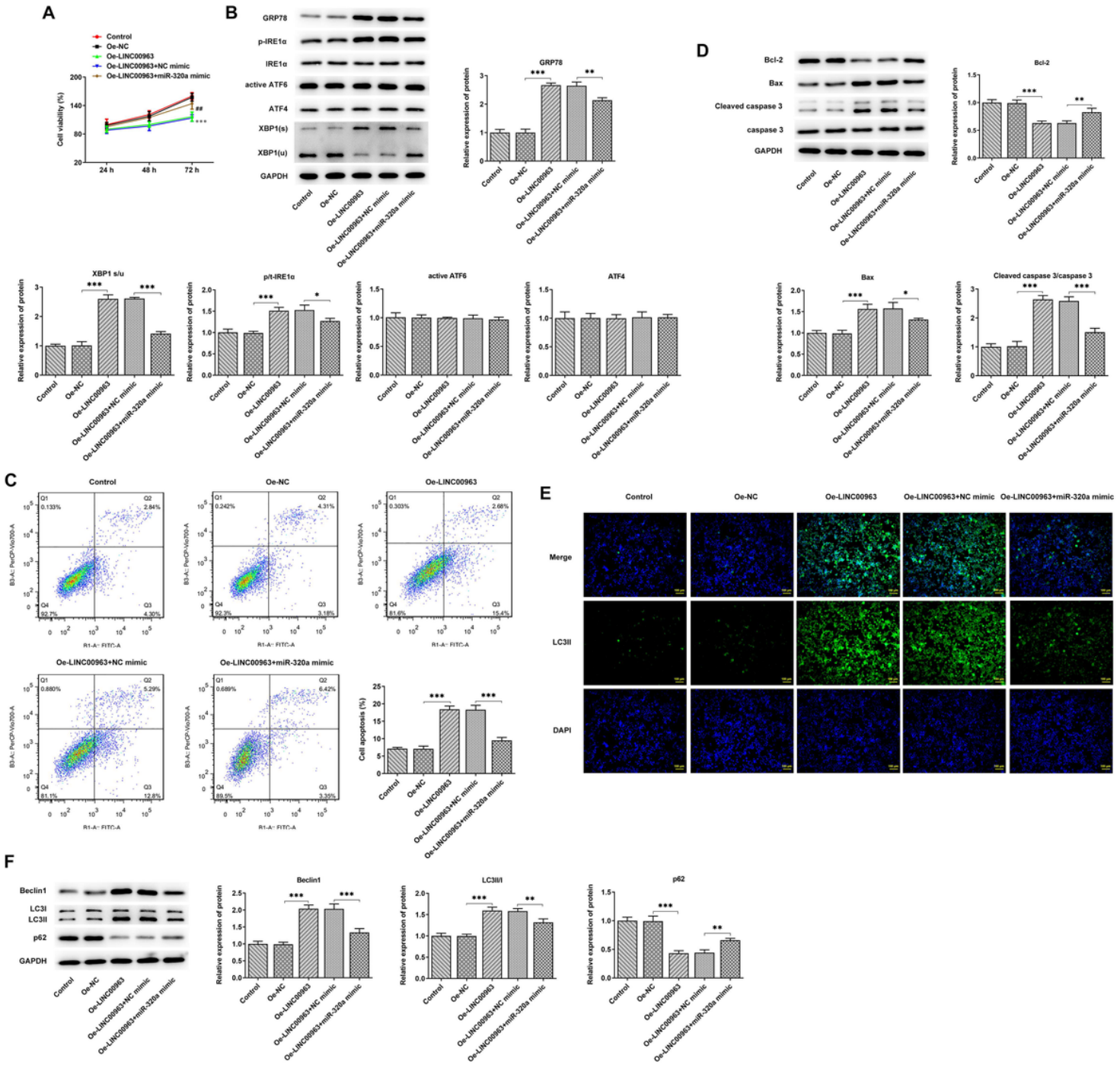

Figure 6

A CCK-8 assay was used to detect cell proliferation in different groups. B Western Blot was employed to detect the expression of ERs markers, including GRP78, p-IRE1α, IRE1α, active ATF6, ATF4, XBP1(s) and XBP1(u). C The analysis of cell apoptosis in each group through Flow cytometry. D Western blot analyzed the expression of Bcl-2, Bax, cleaved caspase 3 and caspase 3. E The expression of LC3 in SUDHL4 cells was analyzed by Immunofluorescence assay. F LINC00963 overexpression regulated the expression of autophagy-related proteins (Beclin1, LC3II, LC3I and p62) through miR-320a.

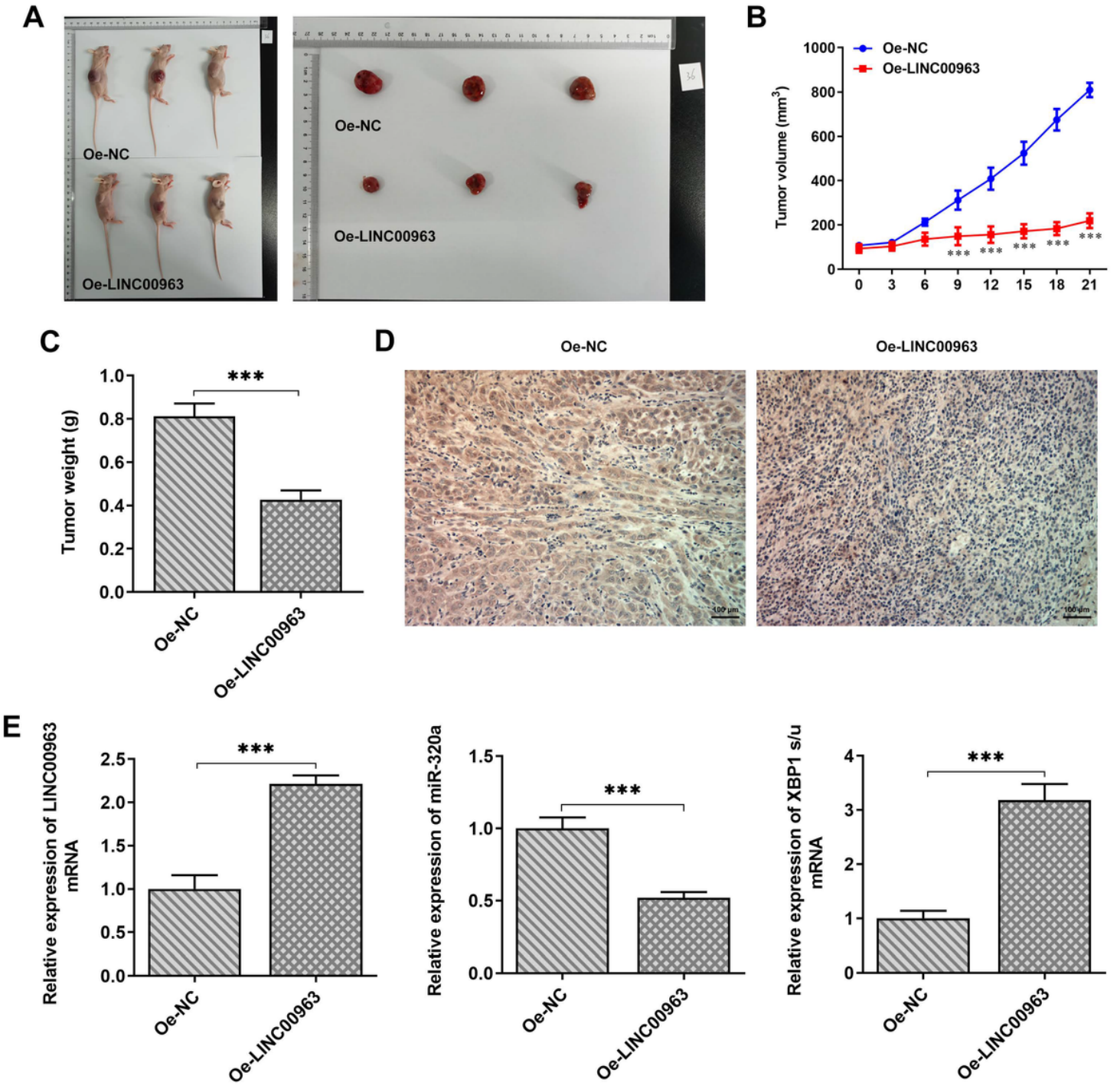

Figure 7

Tumor tissues were taken out, the tumor sizes (A) of each group were photographed and measured and the volume (B) was calculated, and the volume (C) of tumor tissues of each group was statistically analyzed. D The expression of Ki67 was detected through immunohistochemical assay. E Total RNAs from tumor tissues of each group were extracted for RT-qPCR assay to detect the expression of LINC00963, miR-320a, XBP1(s) and XBP1(u) in each group.