JBUON 2021; 26(5): 2033-2039
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Regulatory effects of miR-188-5p/XRCC5 on the progression of natural killer/T-cell lymphoma

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Summary

Purpose: *Natural killer/T cell lymphoma (NKTCL) is a malignant condition. The molecular pathological mechanism of NKTCL has not been well studied. In this article we tried to study the role of microRNA-188-5p (miR-188-5p) in NKTCL.*

Methods: *The expression level of miR-188-5p and XRCC5 was examined by quantitative real-time polymerase chain reaction (qRT-PCR). Cell counting kit-8 (CCK-8) assay and colony formation assay were used to assess the ability of cell proliferation. Dual luciferase reporter assay was used to examine the down-stream target of miR-188-5p. Western blotting was utilized to determine XRCC5 expression level.*

Results: *miR-188-5p was down-regulated in NKTCL. High expression of miR-188-5p accelerated cell proliferation. XRCC5 was one of the down-stream targets. Our data indicated that miR-188-5p suppressed NKTCL progression via regulating XRCC5 expression.*

Conclusions: *This research elucidated that miR-188-5p suppressed tumor progression in NKTCL by regulating XRCC5. Our data may provide more evidence in looking for novel therapeutic targets.*

Key words: *miR-188-5p, proliferation, XRCC5, NKTCL*

Introduction

Natural killer/T cell lymphoma (NKTCL) is a malignant condition associated with Epstein-Barr (EB) virus (EBV) [1], accounting for 2.6-10.7% of all non-Hodgkin's lymphomas. NKTCL is highly invasive, and advanced patients have lost the chance of radiotherapy, making chemotherapy the main clinical treatment. However, patients had poor response to traditional chemotherapy and low long-term survival rate [2]. At present, the molecular pathological mechanism of NKTCL has not been fully elucidated, which may have an important relationship with gene mutation or gene damage.

MicroRNAs (miRNAs/miRs) are non-coding small RNA molecules that can cause RNA silencing, regulate gene expression and function after transcription, and play an important role in cell differentiation, proliferation and apoptosis [3]. Almost all genes encoding proteins are regulated by miRNAs, and studies have shown that miRNA disorders are associated with a variety of diseases, especially malignant tumors [4,5]. Currently, extensive studies have been conducted on the mechanism of miRNA disorders leading to malignant lymphoma, especially B-cell lymphoma [6], but relatively few studies have been conducted on NKTCL.

Our current study was designed to examine the effect of miR-188-5p in NKTCL progression. All data of our research verified that miR-188-5p inhibited tumor progression in NKTCL through regulating XRCC5 expression. Our findings may provide a better understanding of NKTCL occurrence and development.

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Received: 14/06/2021; Accepted: 06/08/2021

Methods

Clinical tissues

This study was approved by the Ethics Committee of Guizhou Provincial People's Hospital. All patients brought into the current research signed the informed consent form. All samples were obtained from NK/T-cell lymphoma patients. A total of 30 paired of NK/T-cell lymphoma and normal tissues were used.

Cell culture

Cell lines involved in this study including 5 NK/T-cell lymphoma cell lines (KHYG-1, NK-92, SNK-1, HANK-1, SNK-6) and natural killer cells (NK) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Rockville, MD, USA) at 37°C with 5% CO_2.

Cell transfection

Oligonucleotides were used for up-regulating miR-188-5p (RiboBio, Guangzhou, China). The plasmid was established from GenePharma (Shanghai, China) and was used to up-regulate XRCC5.

Quantitative real-time polymerase chain reaction (qRT-PCR)

All cDNAs were synthesized *via* Reverse Transcription Kit (TaKaRa, Tokyo, Japan). The expression level of miR-188-5p was determined by PCR (SYBR Green, TaKaRa, Tokyo, Japan). The primer: miR-188-5p: F:5'-CTGGAGATATGGAAGAG-3', R:5'-CATCCCTTGCATG-GTGGAGGG-3'; U6: F:5'-CTCGCTTCGGCAGCACA-3', R:5'-AACGCTTCACGAATTTGCGT-3'; XRCC5: F:5'-TGACTTCCTGGATGCACTAATCGT-3', R:5'-TTGGAGC-CAATGGTCAGTCG-3'; GAPDH: F:5'-CAAGGTCATCCAT-GACAACTTTG-3', R:5'-GTCCACCACCCTGTTGCTGTAG-3'.

Colony formation

Cells (1.0×10^3) were planted into the culture plates and cultured for 2 weeks. Cells on the plates were then washed by phosphate buffer saline (PBS) (Gibco, Rockville, MD, USA) twice and fixed in ice-cold 70% methanol for 15 min. Crystal violet staining solution (Beyotime, Shanghai, China) was used to stain the cell colonies.

Cell-counting kit-8 assay (CCK-8)

Transfected cells were planted into 96-well plates (6×10^3/well) and then CCK-8 solution (Beyotime, Shanghai, China) (10 ul/well) was used to stain cells for 2 h at 37°C. The optical density (OD) value at 450 nm was accessed by a microplate reader (BioTek, Winooski, VT, USA).

Western blotting

Total protein was isolated by radioimmunoprecipitation assay (RIPA) buffer (RiboBio, Guangzhou, China). Protein lysates were then transferred to polyvinylidene fluoride (PVDF) membrane (Roche, Basel, Switzerland). Then, the membrane was immunostained at 4°C by rabbit anti-XRCC5 (1:1000, CST, Danvers, MA, USA) overnight. Rabbit anti-GAPDH (1:5000, CST, Danvers, MA, USA) was taken as a loading control. Protein relative expression level was evaluated by Image J software (NIH, Bethesda, MD, USA).

Statistics

All experiments in this study were performed at least in triplicate. All data recorded were shown as mean ± standard deviation (SD). Student's unpaired t-test was used to undergo statistical analyses. Kaplan-Meier curves and log-rank test were performed for the survival prognosis analysis. P<0.05 was considered to be significant.

Results

miR-188-5p was down-regulated in NKTCL

Through qRT-PCR assay, we verified that miR-188-5p was down-expressed in NKTCL (Figure 1A). miR-188-5p was down-regulated in NKTCL cells when compared with NK cell (Figure 1B). In progression-free survival and overall survival rate analysis, high expression of miR-188-5p indicated relatively good prognosis in NKTCL. In sum, our results showed that miR-188-5p was down-regulated in NKTCL and miR-188-5p over-expression indicated good outcome.

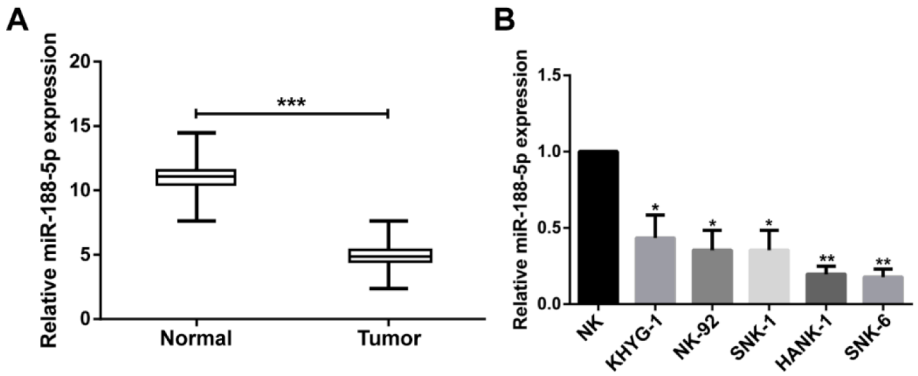

Figure 1. The relative expression level of miR-188-5p in NKTCL. **A:** The relative expression level of miR-188-5p was detected in 30 paired NKTCL cancer and normal tissues. **B:** qRT-PCR was used to verify the expression of miR-188-5p in NKTCL cell lines (*p<0.05, **p<0.01, ***p<0.001, compared to control group. The data are expressed as mean ± SD).

miR-188-5p inhibited cell growth in NKTCL cell lines

For CCK8 and colony formation assay we used SNK-6 and KHYG-1 cell lines for further study. The transfection efficiency was examined by qRT-PCR assay (Figure 2A). CCK8 assay showed that SNK-6 and KHYG-1 cell lines exhibited lower OD values in miR-188-5p mimics group compared with control group (Figure 2B). In colony formation assay, highly expressed miR-188-5p induced less colonies in comparison with the control group (Figure 2C). Hence, we validated that miR-188-5p restrained cell proliferation in NKTCL.

XRCC5 was a down-stream target of miR-188-5p

Several publicly available databases (TargetScan, miRDB and miRWalk) were invovled to predict down-stream target. XRCC5 was found to be one potential target of miR-188-5p. In Figure 3A, we examined the expression level of XRCC5 in miR-188-5p over-expressed cell lines and the expression of XRCC5 was down-regulated. Through qRT-PCR assay, we validated that XRCC5 was highly over-expressed in NKTCL (Figure 3B, 3C). The sequences of potential binding region are shown in Figure 3D. Through dual luciferase reporter assay, miR-188-5p over-expression led to higher luciferase activity with wild 3'-UTR region of XRCC5 (Figure 3E). Moreover, we used the Pearson correlation analysis the results of which indicated that miR-188-5p was negatively correlated with XRCC5 in NKTCL (r^2=0.284, p<0.01). All data revealed that XRCC5 was the down-stream target of miR-188-5p.

miR-188-5p inhibited NKTCL progression through regulating XRCC5

For rescue assay, XRCC5 over-expression plasmid was co-transfected into transfected cell lines. Through qRT-PCR and Western blotting assay, we further revealed that miR-188-5p was negatively correlated with XRCC5 (Figure 4A,4B). As Figure 4C shows, XRCC5 over-expression abolished the effect

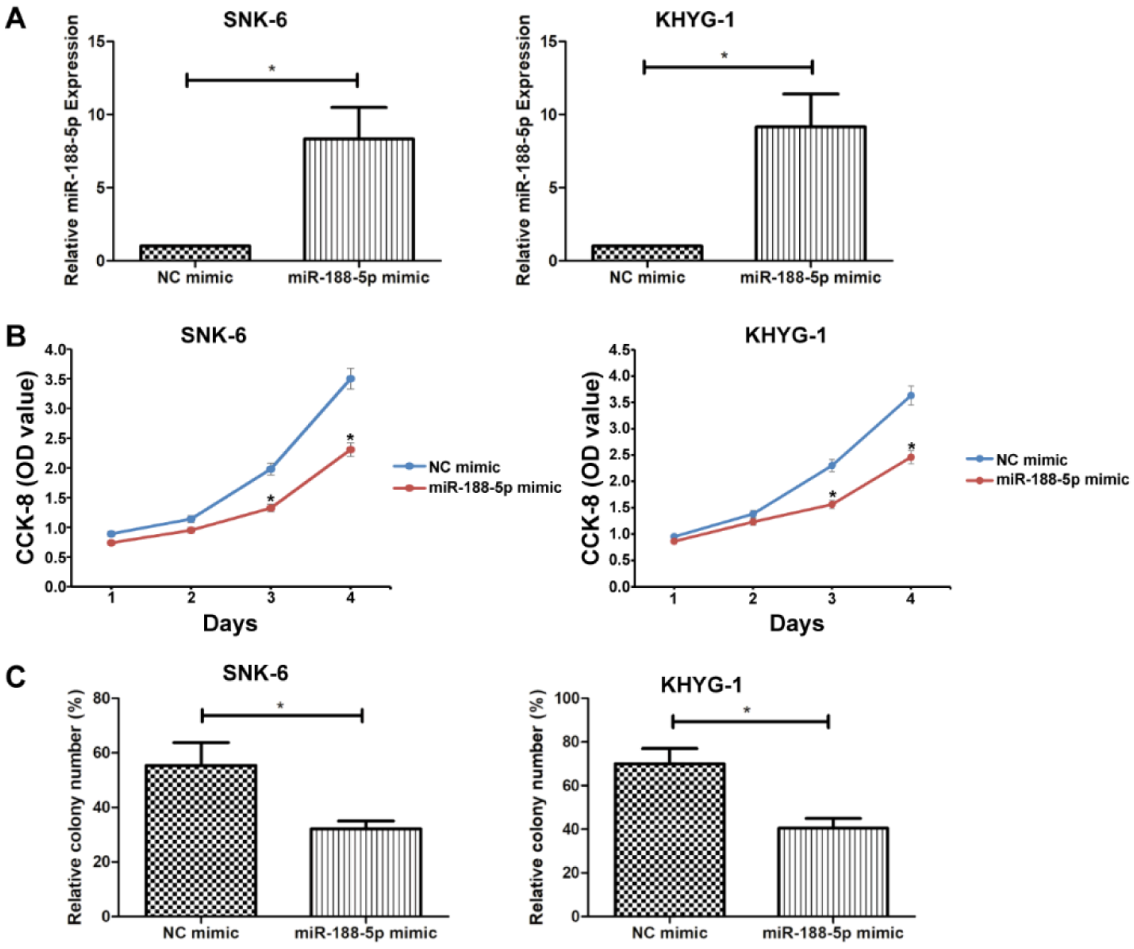

Figure 2. MiR-188-5p over-expressing suppressed cell proliferation in NKTCL cell lines. **A:** Transfection efficiency was detected by qRT-PCR assay. **B:** CCK-8 was used to analyze the cell proliferation ability. **C:** Cell proliferation ability was shown as colony formation ability. (*p<0.05, compared with control group. The data are expressed as mean ± SD).

on cell proliferation caused by up-regulated miR-188-5p. In colony formation assay, high expression of XRCC5 attenuated the inhibition effect caused by miR-188-5p over-expression (Figure 4D). In sum, our results elucidated that miR-188-5p functioned as a tumor suppressor in NKTCL by regulating XRCC5.

Discussion

Lymphomas constitute a malignant proliferative disease originating from cells of the immune system and their progenitors. In the process of human tumor research, lymphomas were the first malignant tumors that could be cured by a combina-

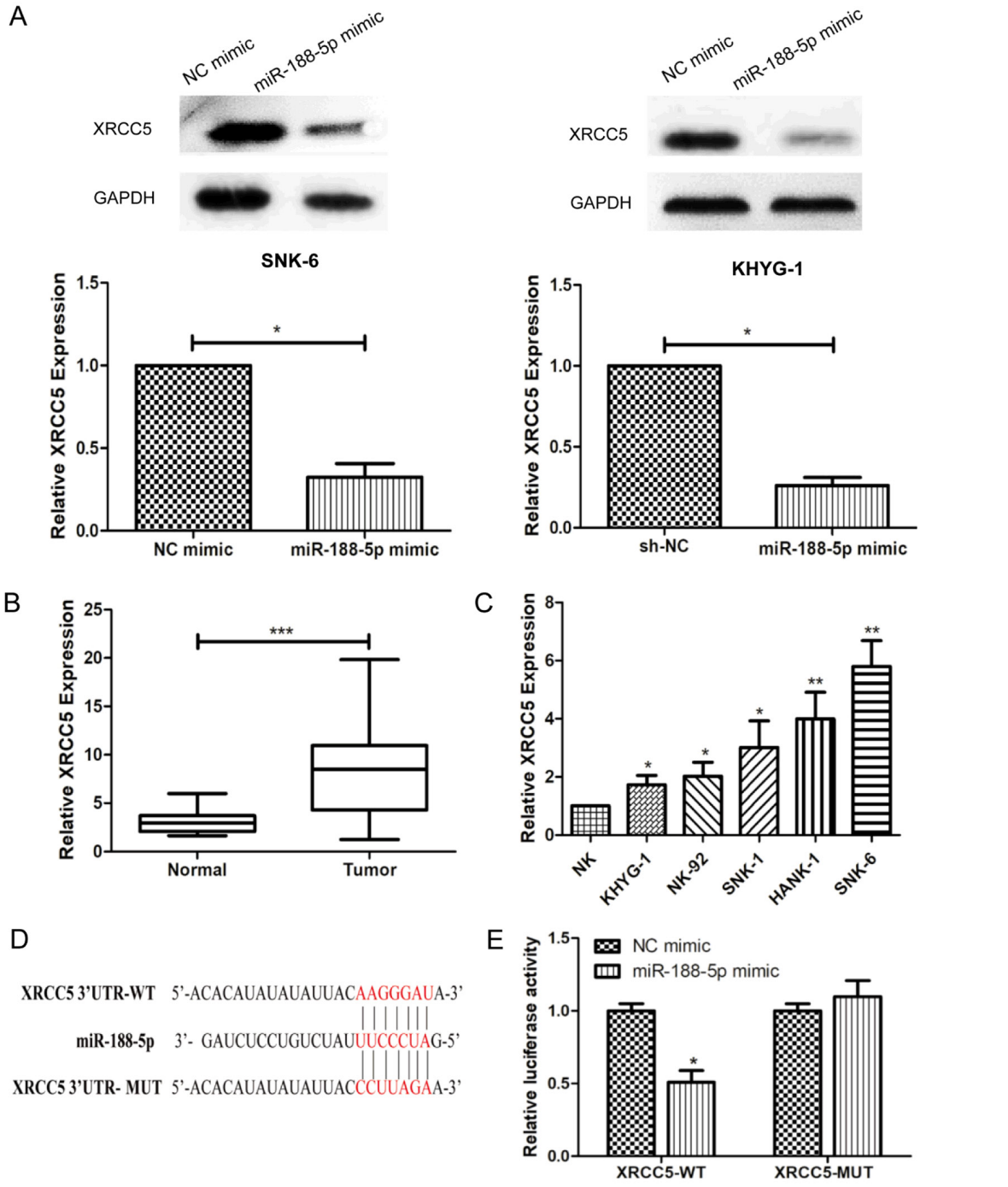

Figure 3. XRCC5 was proved to be the target gene of miR-188-5p. **A:** The protein expression level of XRCC5 in transfected cells. **B:** The expression of XRCC5 in NKTCL and adjacent tissues. **C:** The expression level of XRCC5 in NKTCL cell lines. **D:** The sequence of wild-type and mutant versions in XRCC5 3'-UTR was shown. E: Dual luciferase reporter assay was constructed to prove that miR-188-5p directly bound to the 3'-UTR regions of XRCC5s (*p<0.05, **p<0.01, ***p<0.001, compared with control group. The data are expressed as mean ± SD).

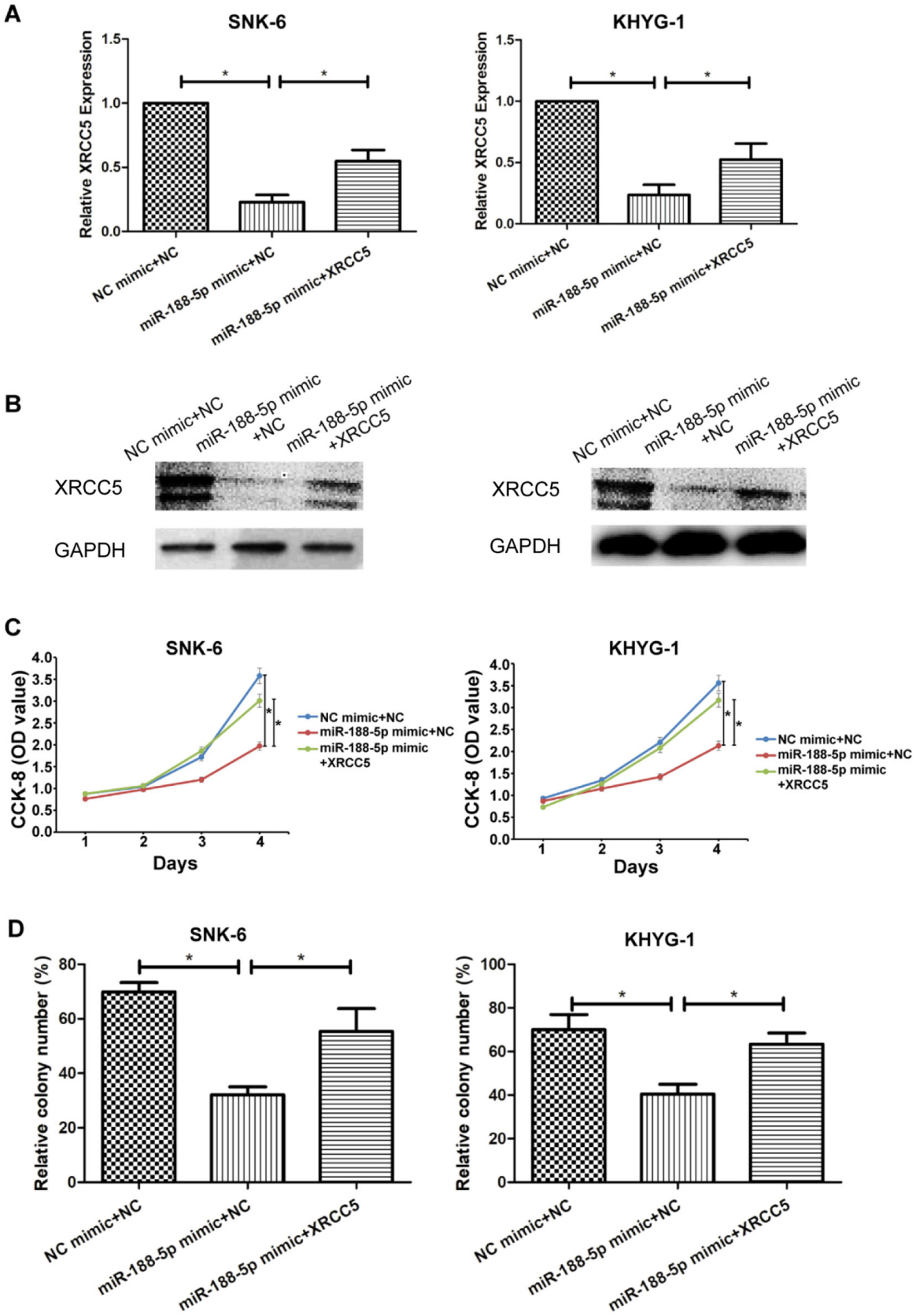

Figure 4. MiR-188-5p functioned as a tumor suppressor by directly targeting XRCC5. **A:** qRT-PCR was used to examine the mRNA level of XRCC5 in cells co-transfected with plasmids and mimics. **B:** Western blotting was used to examine the protein level of XRCC5 in cells co-transfected with plasmids and mimics. **C:** CCK-8 assay was performed to elucidate the effect of XRCC5 on cell proliferation in co-transfected cells. **D:** Colony formation assay was performed to assess the role of XRCC5 in miR-188-5p regulating NKTCL progression (*p<0.05, compared to control group. The data are expressed as mean ± SD).

tion of radiotherapy and chemotherapy [7]. NKTCL is one of the subtypes of non-Hodgkin's lymphoma. NKTCL is characterized by high aggressiveness, rapid disease progression and low survival rate [1].

MicroRNA (miRNA) is a small non-coding RNA molecule, approximately 22 nucleotides in length, which can cause RNA silencing and plays an important role in normal cell differentiation, proliferation and apoptosis [3]. At present, the role of miRNA in NKTCL has been increasingly investigated, and a large number of studies have shown that miRNAs are closely related to the occurrence and development of tumors. Yamanaka et al [8] found that miRNA-21 and miRNA-155 were over-expressed in NKTCL, and activated the AKT signaling pathway *via* down-regulating tumor suppressor genes. Guo et al [9] found that circulating miRNA-221 can be taken as a biomarker in NKTCL. Paik et al [10] found that miRNA-146a suppressed NF-kB by regulating TRAF6 and the expression of TRAF6 was closely related to the prognosis of patients.

XRCC5, also known as Ku80, is encoded by the XRCC5 gene and, together with XRCC6, forms the XRCC5/XRCC6 heterodimer, a DNA-dependent protein kinase complex [11]. Abnormal function of XRCC5 can lead to chromosomal or genomic instability, which can promote tumorigenesis [12]. Therefore, XRCC5 is related to the occurrence and development of tumors to a certain extent. In addition, XRCC5 can also be used as one of the adhesion factors to participate in the adhesion, invasion and migration of tumor cells [13]. Down-regulation of XRCC5 expression can promote the apoptosis of renal cancer cells [14], indicating that XRCC5 may promote the occurrence and development of renal cancer by inhibiting the apoptosis of tumor cells. Down-regulation of XRCC5 expression in esophageal squamous cell carcinoma can inhibit the proliferation of tumor cells [15]. Down-regulation of XRCC5 expression can promote the invasion and migration of breast cancer cells by inhibiting tropomyosin receptor kinase A [16].

Conclusions

Taken together, all data in our study showed that miR-188-5p inhibited tumor progression in NKTCL. This finding may provide novel insight of exploring therapeutic targets for NKTCL.

Conflict of interests

The authors declare no conflict of interests.

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