Article

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Toll-Like Receptor 7 Enhances the Inhibition of Natural Killer Cells on Non-Small Cell Lung Cancer Cell Proliferation by Upregulating the Spliced Form of X-box Binding Protein 1

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Background: Toll-like receptor (TLR) 7 suppresses non-small cell lung cancer (NSCLC) progression and enhances natural killer (NK) cell function. Therefore, this study aimed to elucidate the underlying mechanism of TLR7 enhancing the influences of NK cells on the viability and proliferation of NSCLC cells.

Methods: Identification of NK cells was conducted using flow cytometry. The expression levels of TLR7 and spliced form of x-box binding protein 1 (XBP1s) in lung adenocarcinoma, NK cells, and NSCLC cells were assessed through online website, quantitative reverse transcription polymerase chain reaction (qRT-PCR), and western blot. After overexpressing or silencing TLR7 in NK cells, their impact on the activity and proliferation (immunofluorescence) of NSCLC cells was determined. Furthermore, after transfecting NSCLC cells with TLR7 overexpression plasmid and short hairpin RNA targeting XBP1s (*shXBP1s*), the colony formation capabilities of NSCLC cells and their sensitivity to NK cells were evaluated.

Results: NK cells overexpressing TLR7 exhibited increased viability, high XBP1s expression, and enhanced inhibitory effect on the viability and proliferation of NSCLC cells ($p < 0.01$). Furthermore, TLR7-overexpressing NSCLC cells demonstrated stronger sensitivity to NK cells, but this effect was reversed upon XBP1s silencing ($p < 0.05$). The vitality and proliferation of NSCLC cells were inhibited by TLR7 overexpression and/or XBP1s silencing ($p < 0.05$). TLR7 or XBP1s expression in NSCLC cells was upregulated by TLR7 overexpression or XBP1s silencing ($p < 0.05$).

Conclusion: This study confirmed that TLR7 enhances the inhibitory effect of NK cells on the viability and proliferation of NSCLC cells by upregulating XBP1s expression.

Keywords: non-small cell lung cancer; natural killer cells; toll-like receptor 7; spliced form of x-box binding protein 1

Introduction

Non-small cell lung cancer (NSCLC), the primary malignant tumor, poses considerable human health challenges today [1]. Diagnosing early-stage NSCLC can be challenging, and patients are often diagnosed at an advanced stage, missing the optimal time for surgical intervention. Despite substantial advancements in traditional NSCLC treatment, the therapeutic efficacy remains unsatisfactory [2]. Recently, immunotherapy has emerged as a promising anti-tumor strategy, especially for advanced malignant tumors which cannot be effectively cured by conventional treatments [3]. Natural killer (NK) cells function as the primary effectors of the body's innate immunity, playing an anti-tumor role through immune surveillance and clearance [4]. However, evidence indicates that the function of NK cells is compromised in numerous cancer patients, rendering tumor cells to evade immune surveillance and clearance [5]. Therefore, improving the activity of NK cells and their toxic effect on tumor cells is a crucial research direction to optimize tumor immunotherapy efforts.

Toll-like receptors (TLRs) are widely expressed across various immune cells and participate in the modulation of immune response. Among them, TLR7, located on the surface of the intracellular plasma membrane, possesses a robust immunostimulatory effect. This feature has led researchers to investigate TLR7 agonists as immunostimulants for treating various malignant tumors [6]. Particularly, the TLR7 agonist has been reported to exert a potent anti-tumor immune response in the LC mouse model [7], which can also improve the cytotoxic function of NK cells against tumor cells [8]. However, the mechanism by which TLR7 enhances the anti-tumor effect of NK cells needs to be unveiled.

A spliced form of x-box binding protein 1 (XBP1s), a transcription factor, acts as the therapeutic target across many cancer types. During endoplasmic reticulum stress, the un-spliced XBP1 is activated, cleaved, and transformed into the mature form encoding spliced XBP1 (XBP1s), which is transferred to the nucleus to regulate the transcription of various immune response-related genes [9]. No-

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Table 1. A list of antibodies used in western blot analysis.

Name	Catalog	Molecular weight (kDa)	Dilution	Manufacturer
XBP1s	647501	50	1/500	Biolegend, San Diego, CA, USA
TLR7	ab124928	140	1/1000	Abcam, London, UK
GAPDH	ab8245	37	1/10,000	Abcam, London, UK
Goat anti rabbit	ab205718	—	1/2000	Abcam, London, UK
Goat anti mouse	ab205719	—	1/2000	Abcam, London, UK

XBP1s, x-box binding protein 1; TLR7, toll-like receptor 7; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

tably, existing reports demonstrate that TLR7 can regulate the expression of XBP1s [10] and enhance the vitality and effector function of NK cells [11]. Therefore, it is reasonable to speculate that TLR7 may promote the effector function of NK cells by upregulating XBP1s levels, thereby enhancing the inhibition function of NK cells on NSCLC cells.

Materials and Methods

Cell Culture

Human NK cells (CP-H168, Procell, Wuhan, China) were cultured in a specific medium (CM-H168, Procell, Wuhan, China). However, human non-small cell lung cancer (NSCLC) cell lines A549 and H1299 (iCell-h011/iCell-h153, iCell, Shanghai, China) were cultivated in RPMI-1640 Medium (iCell-0002) replete with 10% fetal bovine serum (FBS, iCell-0500) and 1% Penicillin-Streptomycin Solution (iCell-15140-122). The cultures were maintained in a humid environment at 37 °C and 5% CO_2. The NK cells and NSCLC cells were co-cultured at a 1:1 ratio for 4 hours, following the previously described method [12]. The cells underwent routine short tandem repeat (STR) identification and mycoplasma testing, confirming their contamination-free status.

Flow Cytometry

NK cells were incubated with anti-CD3 (317319, BioLegend, San Diego, CA, USA) and anti-CD56 (362502, BioLegend, San Diego, CA, USA) antibodies at room temperature for 20 minutes. Finally, the percentage of $CD3^{-}CD56^{+}$ cells was analyzed using a flow cytometer (FACSCanto II, BD Biosciences, San Jose, CA, USA) [13].

Cell Transfection

TLR7 overexpression plasmid (HG11204-M) and the empty vector (pMD18-T Simple Vector) were obtained from SinoBiological (Beijing, China). The coding sequence of TLR7 is provided in the **Supplementary Materials**. Short hairpin RNA (shRNA) targeting *TLR7* or *XBP1s* (*shTLR7*, target sequence: GAAATGAGATTGCCCATATTT) (*shXBP1s*, target sequence: GAACAGCAAGTGGTAGATTTA) and the negative control (shNC) were synthesized by VectorBuilder (Guangzhou, China). The cells were seeded in a 24-well plate and subsequently transfected with plasmid using a Sinofection reagent (Sinotransfection, Sinobiology, Beijing, China). After 8 hours of transfection, cells were cultured in a fresh medium for additional 16 hours. Transfection efficiency was determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Grouping

The experimental procedure consisted of three parts. Firstly, TLR7 was either overexpressed or silenced in NK cells, and the viability and expression of XBP1s were evaluated. Secondly, NSCLC cells were treated with NK cells with either TLR7 overexpression or knockdown, followed by the detection of viability and expression of ki67 in NSCLC cells. Lastly, NSCLC cells co-transfected with TLR7 overexpression plasmid and *shXBP1s* experienced treatment of normal NK cells or not.

Western Blot Analysis

Total protein was extracted from NK cells or NSCLC cells employing RIPA buffer (SBJ-0995, Senbeijia, Nanjing, China) and subsequently quantified using Protein Quantitative Kit (JN24501, Jining Shiye, Jining, China). After this, they were resolved through SDS-PAGE (SBJ-0899, Senbeijia, Nanjing, China) and were transferred onto a PVDF membrane. After blocking with 5% Bovine Serum Albumin (BSA) (G5001, Servicebio, Wuhan, China), the membrane was sequentially probed with primary and secondary antibodies (Table 1). The protein bands were visualized utilizing an ECL luminescence reagent (AL008-1, ACE Biotechnology, Nanjing, China), and images of protein bands were captured through a gel imaging system (610020-9Q, Qinxiang, Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

qRT-PCR

Total RNA was isolated from NK or NSCLC cells using an RNA purification kit (B0004D, EZB, Beijing, China) and underwent reverse transcription employing a cDNA Synthesis Kit (EZB-RT2, EZB, Beijing, China). qRT-PCR was performed utilizing SYBR qPCR mix (A0001, EZB, Beijing, China) on the qPCR System (ABI QuantStudio

Table 2. A list of primers used in qRT-PCR.

Genes	5′ → 3′
TLR7 F	AGCGTCCTTTCACAGACTGG
TLR7 R	TTTTTACACGGCGCACAAGG
XBP1s F	ACGGGACCCCTAAAGTTCTG
XBP1s R	TGCACGTAGTCTGAGTGCTG
GAPDH F	CCATGGGGAAGGTGAAGGTC
GAPDH R	AGTGATGGCATGGACTGTGG

Abbreviation: F, Forward; R, Reverse; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

6 Flex, Thermo Fisher Scientific, Waltham, MA, USA). The expression level of RNA was quantified employing the $2^{-\Delta\Delta Ct}$ method [14] and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The list of primers used in qRT-PCR is shown in Table 2.

Cell Counting Kit-8 (CCK-8) Assay

The viability of NK and NSCLC cells was assessed using a CCK-8 kit (GK10001, GLPBIO, Shanghai, China). For this purpose, the cells were seeded in a 96-well plate at a density of 3000 cells/well and incubated at 37 °C for 24 hours. After this, the culture medium was discarded, and the cells underwent another 2-hour incubation with CCK-8 solution. The absorbance was assessed at 450 nm using a microplate reader (CMaxPlus, MD, Shanghai, China), and cellular viability was determined as follows: Cell viability = (absorbance at 24 hours – absorbance at 0 hours)/absorbance at 0 hours × 100%.

Bioinformatics Analysis

The expression levels of XBP1 and TLR7 in lung adenocarcinoma (LUAD) samples (n = 515) and normal samples (n = 59) were predicted using The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) database.

Colony Formation Assay

After 2-week cell culture in a 6-well plate (600 cells/well), cells were fixed utilizing 4% paraformaldehyde (abs9179, absin, Shanghai, China), followed by staining with crystal violet dye (abs817172, absin, Shanghai, China). The cells were observed using an optical microscope (N300M, Yongxin, Ningbo, China), and images of the colonies containing >50 cells were captured. Finally, the images were analyzed utilizing Image J software (1.8.0 version, National Institutes of Health, Bethesda, MA, USA).

Immunofluorescence

Cell suspension was added to a cell slide, which was then plated in a 6-well plate. The cells were subjected to fixation and blocking, followed by incubation with Alexa Fluor® 647-labeled anti-Ki67 antibody (ab196907, Abcam, London, UK) and DAPI (SBJ-0221, Senbeijia, Nanjing, China). After mounting with an Anti-fade mounting medium (G1401, Servicebio, Wuhan, China), the cells were observed employing a laser confocal microscope (LSM880, Zeiss, Oberkohen, Germany).

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). The measurement data were expressed as the mean ± standard deviation. An independent sample *t*-test was used for two-group comparisons. Furthermore, multi-group comparisons were conducted utilizing one-way analysis of variance (ANOVA) followed by the Tukey test for post-hoc analysis. Statistical significance was achieved at a p-value < 0.05.

Results

TLR7 Facilitates NK Cell Viability and XBP1 Expression

We identified $CD3^{-}CD56^{+}$ NK cells using flow cytometry (Fig. 1A). The expression levels of XBP1s and TLR7 proteins were significantly elevated in NK cells following stimulation with interleukin (IL)-12 and IL-18 (Fig. 1B,C, $p < 0.05$). To assess the role of TLR7 in NK cells, TLR7 was either overexpressed or knocked down in these cells (Fig. 1D, $p < 0.01$). Relative to control NK cells, the activity of NK cells with TLR7 overexpression was promoted, while that of NK cells with TLR7 knockdown was inhibited (Fig. 1E, $p < 0.05$). Moreover, the protein levels of XBP1s were substantially increased in TLR7-overexpressing NK cells but decreased in NK cells with TLR7 knockdown (Fig. 1F,G, $p < 0.001$).

Overexpression of TLR7 Enhances the Inhibitory Effect of NK Cells on the Viability and Proliferation of NSCLC Cells

NSCLC cells received treatment of normal NK cells, NK cells transfected with vector or NK cells with TLR7 overexpression/knockdown. The viability of NSCLC cells was decreased after NK cell treatment, and this decrement was further deepened by the therapy involving TLR7-overexpressing NK cells (Fig. 1H,I, $p < 0.01$). Immunofluorescence staining of ki67 revealed that the inhibitory effect of NK cells on the proliferation of NSCLC cells was enhanced by TLR7 overexpression and was partially dampened by TLR7 knockdown (Fig. 1J–L, $p < 0.001$).

Overexpression of TLR7 in NSCLC Cells Potentiates the Inhibitory Effect of NK Cells on the Viability and Proliferation of NSCLC Cells

We observed that TLR7 level decreased while XBP1 increased in LUAD (Fig. 2A, $p < 0.001$). To evaluate the effect of TLR7/XBP1s on NSCLC cells, we overexpressed TLR7 and silenced XBP1s in NSCLC cells (Fig. 2B–E, p

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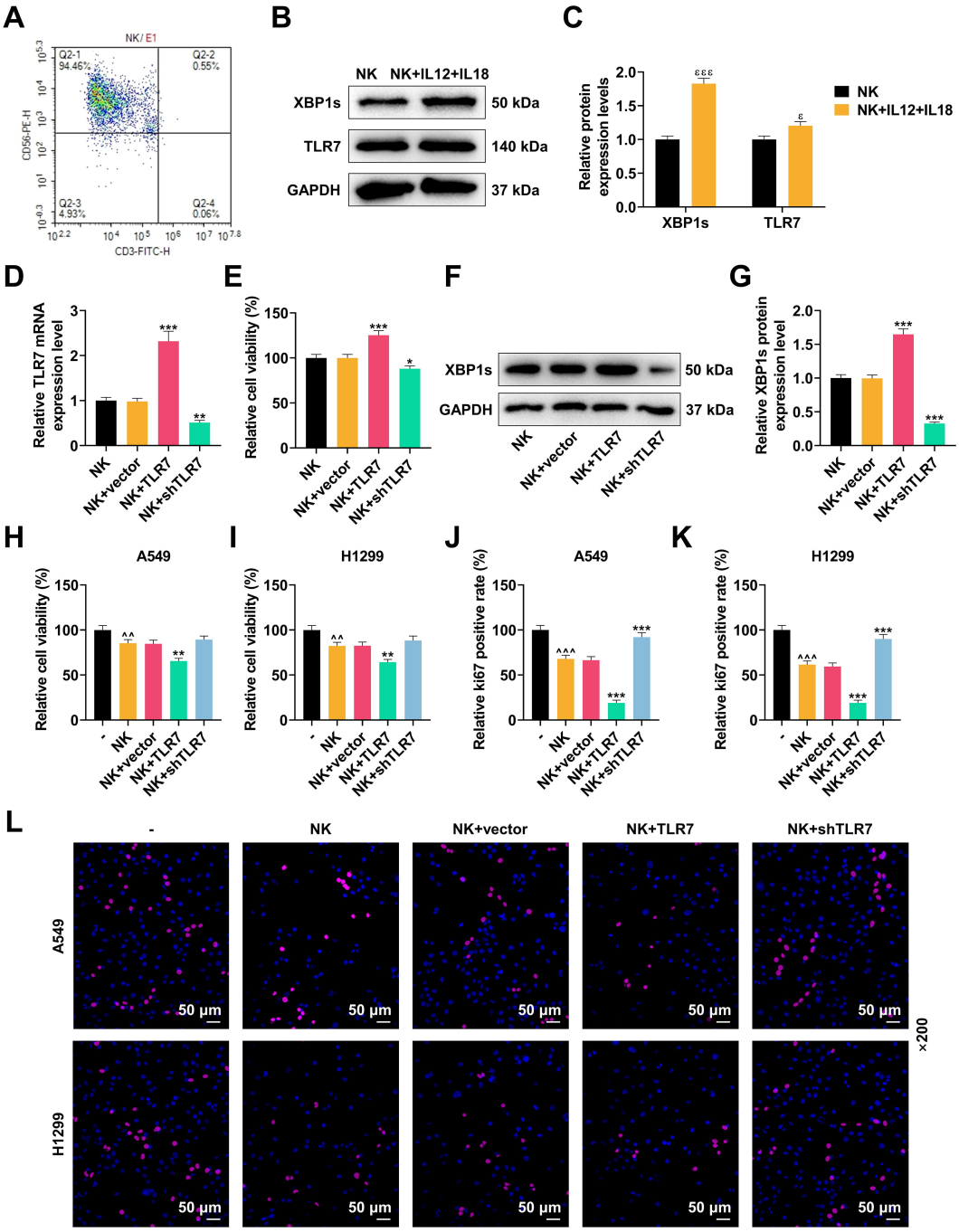

Fig. 1. TLR7 enhances the inhibitory effect of NK cells on NSCLC cells. (A) Identification of NK cells (flow cytometry). (B,C) Expression levels of XBP1s and TLR7 in NK cells stimulated with IL-12 and IL-18 (western blot analysis, GAPDH as an internal control). (D–G), NK cells transfected with TLR7 overexpression plasmid or *shTLR7*. (D) Transfection efficiency of TLR7 overexpression plasmid and *shTLR7* in NK cells (qRT-PCR, GAPDH as an internal control). (E) The viability of transfected NK cells (CCK-8 assay). (F,G) XBP1s protein levels in transfected NK cells (western blot analysis, GAPDH as an internal control). (H–L) NSCLC cells (A549 or H1299) were treated with NK cells or transfected NK cells, with NSCLC cells alone as the control group (– group). (H,I) NSCLC cell viability (CCK-8). (J–L) ki67 expression in NSCLC cells (immunofluorescence) (magnification, 200×). Scale bar = 50 μm. $^{\varepsilon}p < 0.05$, $^{\varepsilon\varepsilon\varepsilon}p < 0.001$ vs. NK group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. NK + Vector group. $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. – group. Quantified values obtained from three independent experiments were presented as the mean ± standard deviation. N = 3. NK, natural killer; NSCLC, non-small cell lung cancer; XBP1s, x-box binding protein 1; TLR7, toll-like receptor 7; IL, interleukin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CCK-8, cell counting kit-8; qRT-PCR, quantitative reverse transcription polymerase chain reaction; *shTLR7*, short hairpin RNA targeting TLR7.

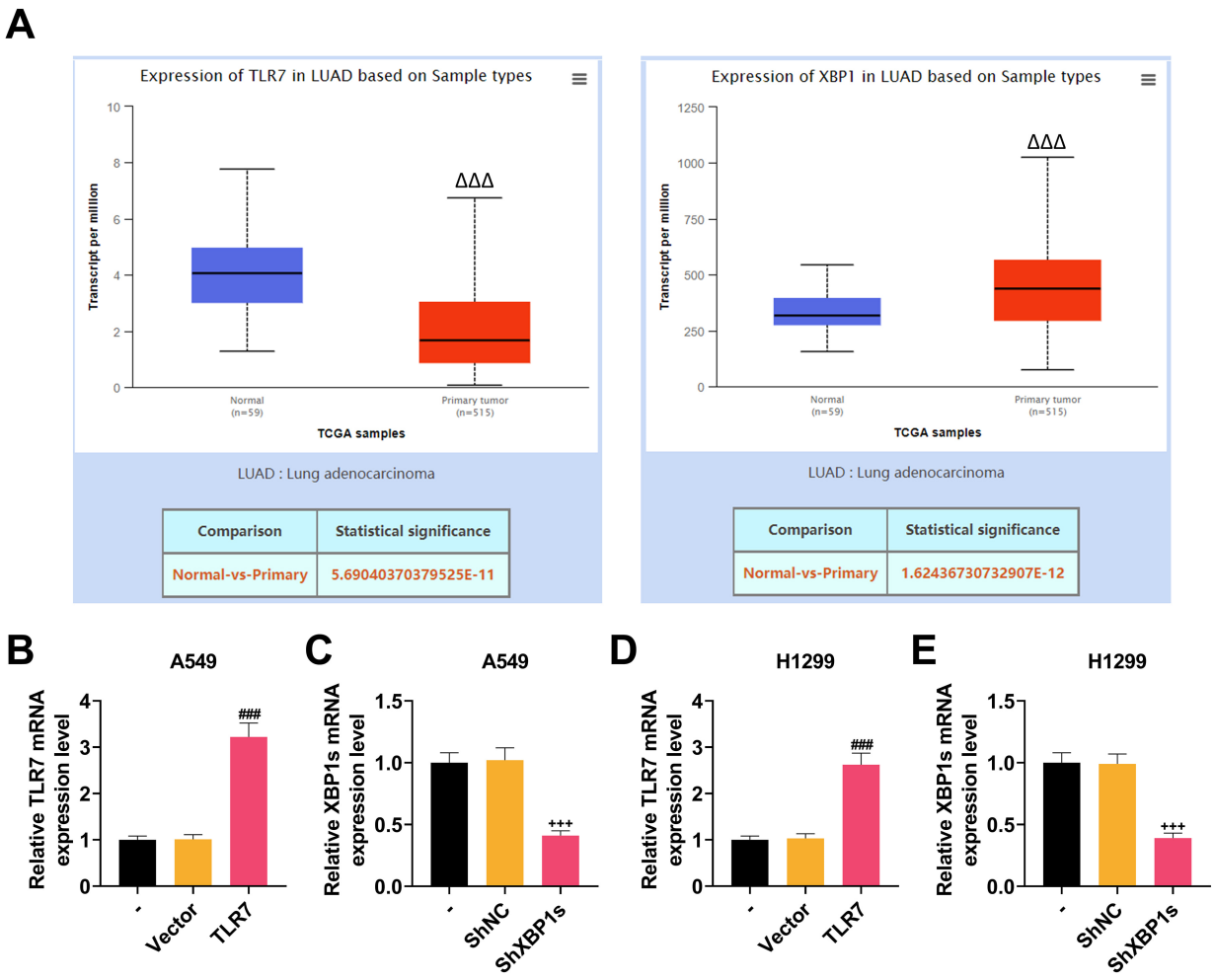

Fig. 2. Downregulation of TLR7 and upregulation of XBP1 in LUAD. (A) Prediction of XBP1 and TLR7 expressions in LUAD (n = 515) and normal samples (n = 59) (The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/)). (B–E) Transfection efficiency of TLR7 overexpression plasmid or *shXBP1s* in NSCLC cells (A549 and H1299) (quantitative reverse transcription polymerase chain reaction, GAPDH as an internal control). $^{\Delta\Delta\Delta}p < 0.001$ vs. Normal group. $^{\#\#\#}p < 0.001$ vs. Vector group. $^{+++}p < 0.001$ vs. shNC group. Quantified values obtained from three independent experiments were presented as the mean ± standard deviation. N = 3. NSCLC, non-small cell lung cancer; XBP1s, x-box binding protein 1; TLR7, toll-like receptor 7; LUAD, lung adenocarcinoma; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; shNC, shRNA negative control.

< 0.001). Transfected NSCLC cells were treated with NK cells, which led to a significant reduction in NSCLC cell viability following TLR7 overexpression (Fig. 3A,B, $p <$ 0.001). Conversely, XBP1s silencing exerted no direct impact on NSCLC cell viability but effectively reversed the inhibitory effect of TLR7 overexpression (Fig. 3A,B, $p <$ 0.05). Additionally, TLR7 overexpression resulted in alleviated ki67 expression in NSCLC cells (Fig. 3C–E, $p <$ 0.001). Similarly, XBP1s silencing did not affect ki67 expression in NSCLC cells but partially reversed the inhibiting effect of TLR7 overexpression (Fig. 3C–E, $p < 0.001$).

Overexpression of TLR7 or Silencing of XBP1s Reduces Colony Formation Ability and Vitality of NSCLC Cells

We co-transfected TLR7 overexpression plasmid/empty vector and *shXBP1s*/shNC into NSCLC cells, and then evaluated the biological behavior of NSCLC cells. TLR7 overexpression or XBP1s silencing both reduced the clonogenic ability and inhibited the viability of NSCLC cells. However, their combination further enhanced the inhibitory effect on cell clonogenic ability and viability (Fig. 4A–E, $p < 0.001$). Furthermore, overexpression of TLR7 augmented XBP1s levels in NSCLC cells, but this effect was reversed by *shXBP1s* transfection (Fig. 5A–D, $p < 0.01$). Similarly, XBP1s silencing partly increased TLR7 expression, which was further enhanced by transfection of TLR7 overexpression plasmid (Fig. 5A–D, $p < 0.05$).

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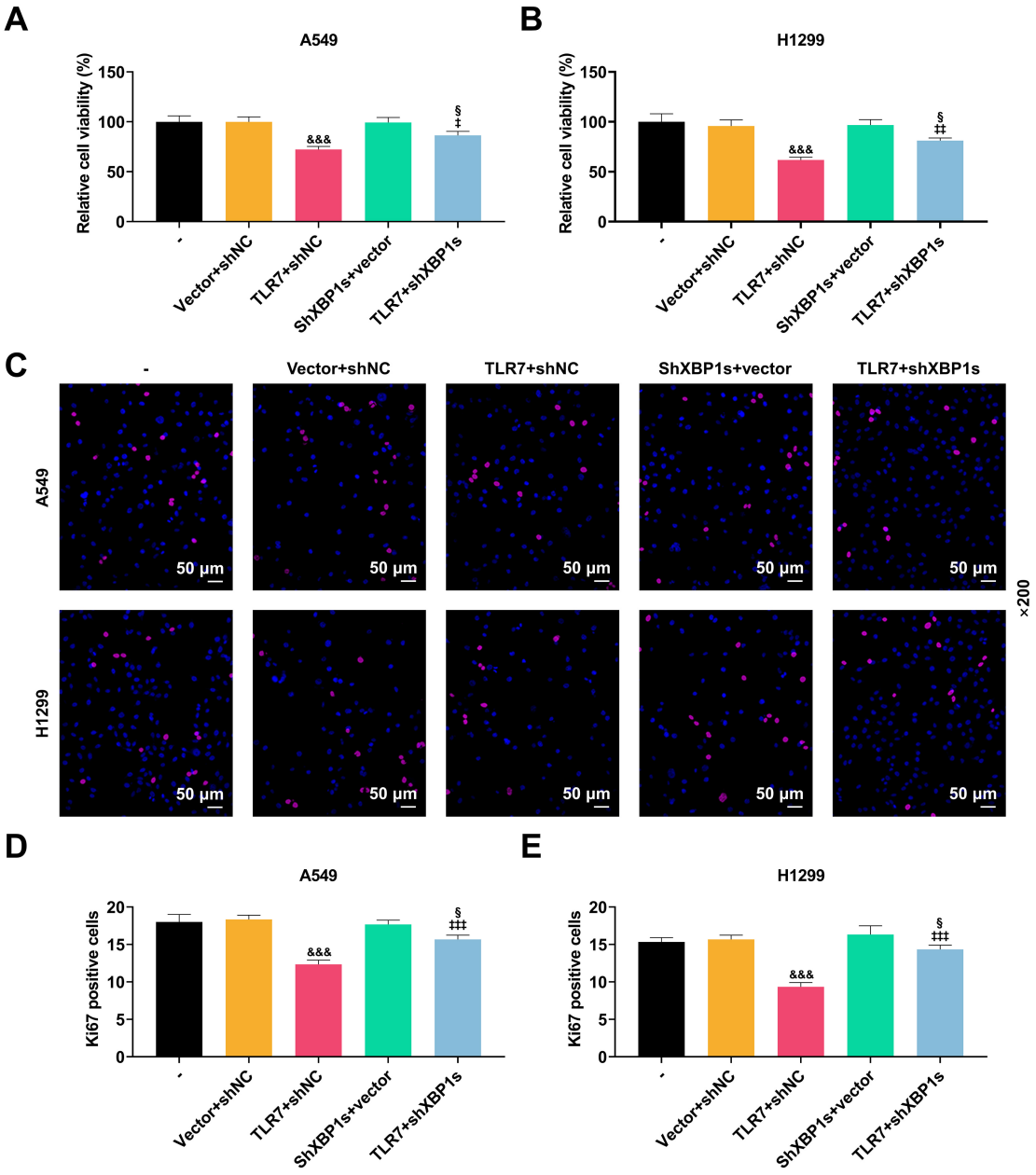

Fig. 3. TLR7 overexpression in NSCLC cells enhances the inhibitory effect of NK cells on the vitality and proliferation of NSCLC cells. NSCLC cells co-transfected with TLR7 overexpression plasmid or empty vector and *shXBP1s* or shNC were treated with normal NK cells (untransfected). (A,B) NSCLC cell viability (cell counting kit-8). (C–E) ki67 expression in NSCLC cells (immunofluorescence) (magnification, 200×). Scale bar = 50 μm. $^{\&\&\&}p < 0.001$ vs. Vector + shNC group. $^{‡}p < 0.05$, $^{‡‡}p < 0.01$, $^{‡‡‡}p < 0.001$ vs. TLR7 + shNC group. $^{§}p < 0.05$ vs. *shXBP1s* + vector group. Quantified values obtained from three independent experiments were presented as the mean ± standard deviation. N = 3. NSCLC, non-small cell lung cancer; XBP1s, x-box binding protein 1; TLR7, toll-like receptor 7; NC, negative control; *shXBP1s*, short hairpin RNA targeting *XBP1s*.

Discussion

At present, the obstacle of cancer treatment is that tumor cells have high inhibitory effects on innate immunity and adaptive immunity. While adaptive immunity plays a pivotal role in tissue tumor progression, it is noteworthy that NK cells within the innate immune system face relatively reduced inhibition by tumor cells [15]. Additionally, activated innate immunity can prolong the duration of adaptive immune responses [16]. Therefore, the therapy targeting NK cells is a promising immunotherapy strategy.

Numerous preclinical studies have used the activation potential of TLR7 within NK cells to improve anti-tumor immunity, and the underlying mechanism involves TLR7 triggering the cytotoxic activity of immune effector cells to eliminate emerging tumors [17,18]. Additionally, the

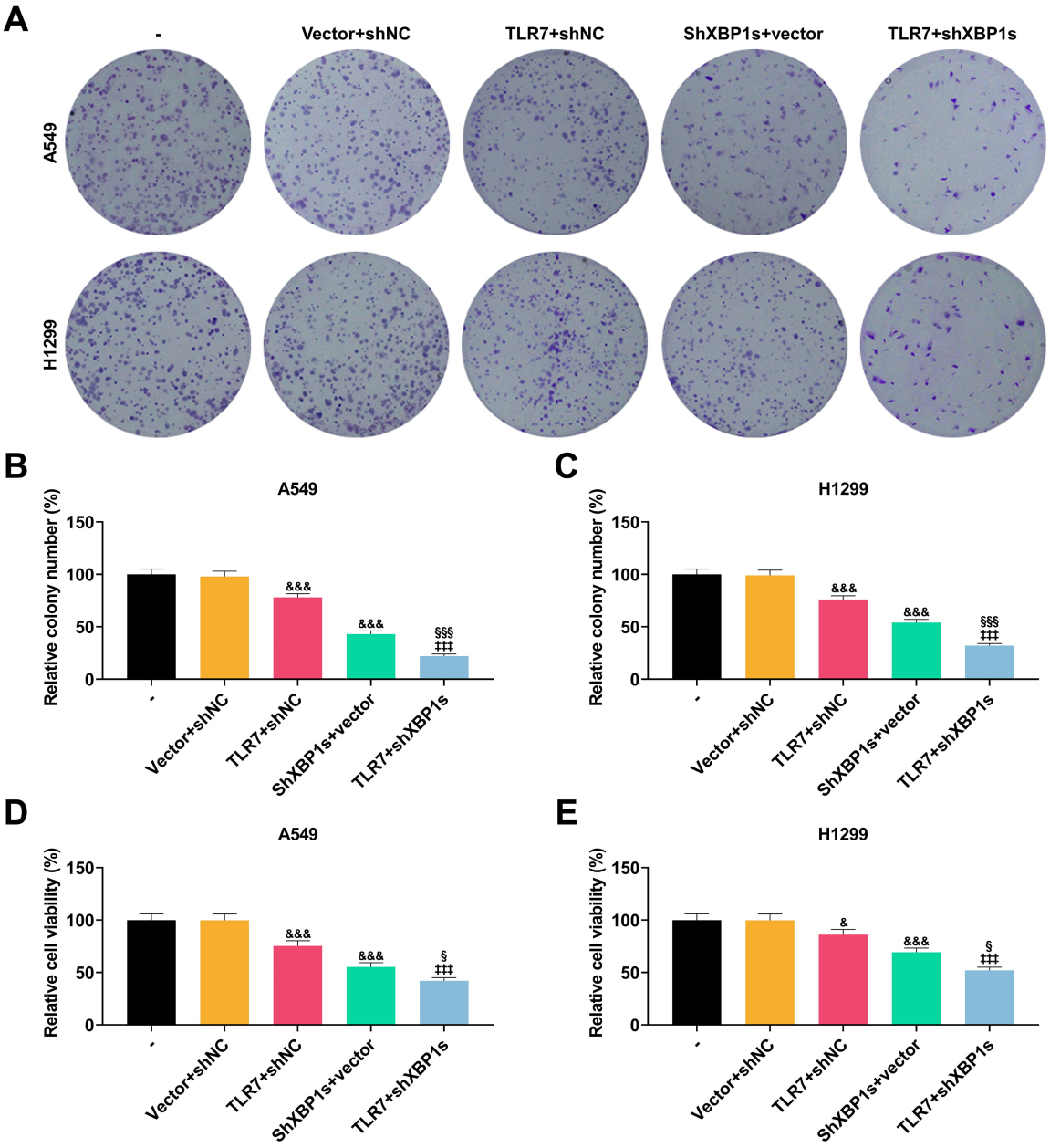

Fig. 4. TLR7 overexpression or XBP1s silencing reduces colony formation ability and vitality of NSCLC cells. NSCLC cells were co-transfected with TLR7 overexpression plasmid/empty vector and *shXBP1s*/shNC. (A–C) NSCLC cell colony formation ability (colony formation assay). (D,E) NSCLC cell viability (cell counting kit-8). $^{\&}p < 0.05$, $^{\&\&\&}p < 0.001$ vs. Vector + shNC group. $^{‡‡‡}p < 0.001$ vs. TLR7 + shNC group. $^{\S}p < 0.05$, $^{\S\S\S}p < 0.001$ vs. *shXBP1s* + vector group. Quantified values obtained from three independent experiments were presented as the mean ± standard deviation. N = 3. NSCLC, non-small cell lung cancer; NC, negative control; TLR7, toll-like receptor 7; NC, negative control; *shXBP1s*, short hairpin RNA targeting XBP1s.

TLR7 agonist Imiquimod has been utilized to treat cancer [19], and Resiquimod, another TLR7 agonist, has indicated a potent anti-tumor effect in NSCLC [20]. Consistent with the previous studies, our finding demonstrated that overexpression of TLR7 in NK cells not only suppresses viability but also potentiates their inhibitory effect on NSCLC cell viability and proliferation. This observation indicates that TLR7 overexpression enhances the inhibitory function of NK cells against NSCLC cells.

However, the role of TLR7 in NSCLC remains controversial [21]. On the one hand, in the lung cancer (LC) model, TLR7/8 agonist can activate tumor-specific T cells and NK cells, thereby activating an anti-tumor immune response [7]. For example, the TLR7 agonist Imiquimod exerts anti-tumor effects by suppressing the production of angiogenic mediators in NSCLC cells and maintaining the transduction of pro-apoptotic signals [22]. On the other hand, TLR7 can stimulate tumor cells to enhance NSCLC

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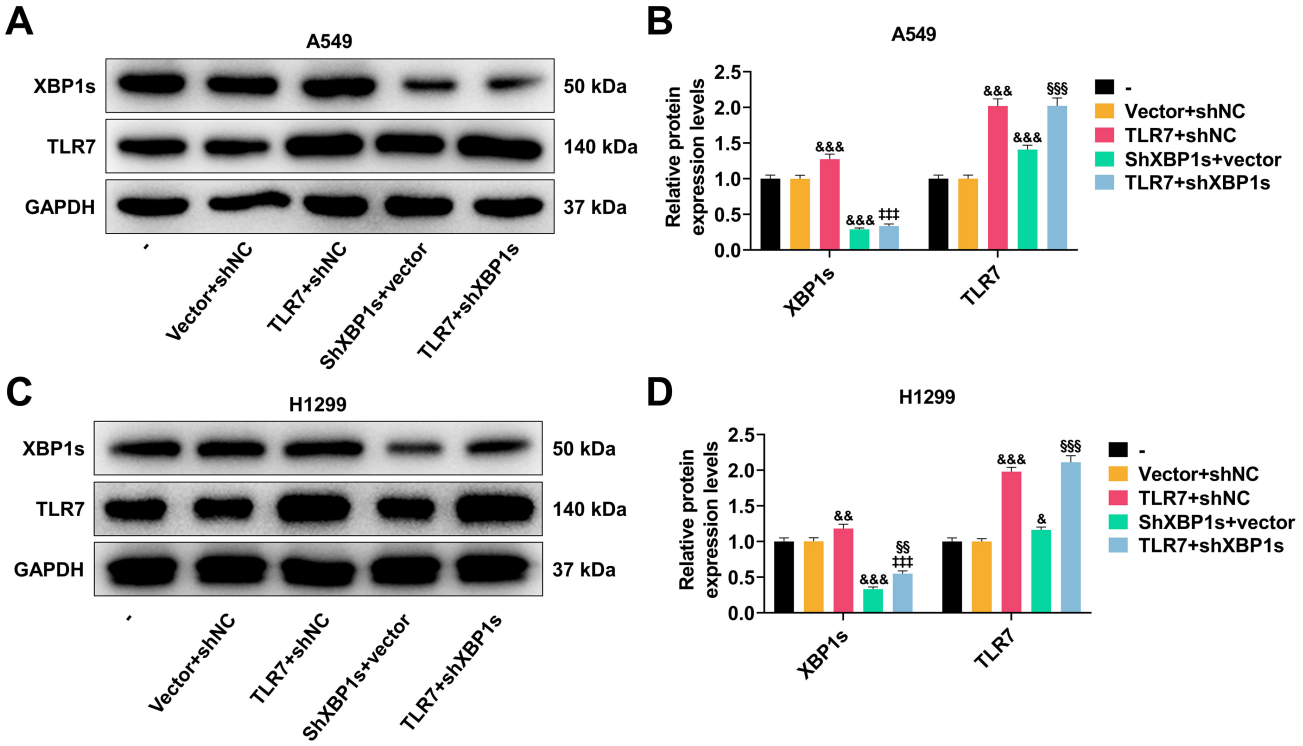

Fig. 5. The protein levels of XBP1s and TLR7 in NSCLC cells. NSCLC cells were co-transfected with TLR7 overexpression plasmid/empty vector and *shXBP1s*/shNC. (A–D) XBP1s and TLR7 protein levels (western blot analysis, GAPDH as an internal control). $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. Vector + shNC group. $^{‡‡‡}p < 0.001$ vs. TLR7 + shNC group. $^{§§}p < 0.01$, $^{§§§}p < 0.001$ vs. *shXBP1s* + vector group. Quantified values obtained from three independent experiments were presented as the mean ± standard deviation. N = 3. NSCLC, non-small cell lung cancer; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; CCK-8, cell counting kit-8.

development. Chatterjee *et al*. [23] reported that TLR7 promotes the development and chemotherapy resistance of NSCLC by activating the NF-κB pathway. In our study, after overexpression of TLR7 in NSCLC cells, cells vitality and proliferation were decreased, but their sensitivity to NK cells was increased, signifying an anti-tumor role for TLR7 in NSCLC cells. Regarding the different effect of TLR7, we speculated that it might depend on tumor background and the signaling pathway for TLR7 activation.

In this study, we confirmed that TLR7 enhances the cytotoxic function of NK cells against NSCLC cells by upregulating the XBP1s level. After splicing and nuclear translocation, XBP1 induces the transcription of different target genes, with the type of target genes depending on the specific stimuli and cell type [9]. In NK cells, IL-12 and IL-18 stimulation or IL-15 stimulation leads to the increase of XBP1s expression in NK cells, promotes cell survival and enhances cell function [11,24]. In contrast, in LC cells, XBP1s overexpression correlates with a poor prognosis [25]. Furthermore, XBP1s can induce endoplasmic reticulum stress, protecting cancer cells from apoptosis and promoting NSCLC metastasis [26,27]. Similar to the previous studies, our study indicated that silencing of XBP1s in NSCLC cells inhibited NSCLC cell vitality and proliferation, manifesting that XBP1s play a pro-cancer role in NSCLC cells. Interestingly, TLR7 overexpression enhanced the inhibitory effect of normal NK cells on NSCLC cell viability and proliferation. However, this effect was reversed upon XBP1s silencing, suggesting that TLR7 in NSCLC cells enhanced the sensitivity of NSCLC cells to NK cells by increasing XBP1s levels. A study exploring the sensitivity of multiple myeloma (MM) cells to bortezomib (BTZ) showed that BTZ-resistant MM cells exhibited a lower level of XBP1s [28]. Therefore, although the increase of XBP1s expression in NSCLC cells may protect them from apoptosis, the effect of intercellular communication cannot be ruled out [29].

Conclusion

In summary, this study corroborated that TLR7 enhances the inhibitory effect of NK cells on NSCLC cell viability and proliferation by upregulating XBP1s expression, offering new insights for the treatment of LC. To validate these findings and enhance the robustness of this study, additional investigation involving animal-based experiments is required.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

Substantial contributions to conception and design: CW. Data acquisition, data analysis and interpretation: LC. Drafting the article or critically revising it for important intellectual content: both authors. Final approval of the version to be published: both authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: both authors.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.415.

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