Epigenetic Silencing of Tumor Suppressor lncRNA NKILA: Implication on NF-κB Signaling in Non-Hodgkin's Lymphoma

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Research

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Abstract

Background: *NKILA,* localized to 20q13.31, is a negative regulator of NF-κB signaling implicated in carcinogenesis. As a CpG island is embedded in the promoter region of *NKILA*, we hypothesized that *NKILA* is a tumor suppressor lncRNA reversibly silenced by promoter DNA methylation in non-Hodgkin's lymphoma (NHL).

Results: By pyrosequencing-verified methylation-specific PCR (MSP), *NKILA* was unmethylated in normal healthy controls, including 10 peripheral blood buffy coats and 11 normal tonsils tissue, but completely methylated in one (10%) NHL cell line SU-DHL-6. Among the lymphoma cell lines, by semi-quantitative RT-PCR, methylation of *NKILA* was inversely correlated with its expression. In the completely methylated SU-DHL-6 cells, hypomethylation treatment with 5-Aza-2'-deoxycytidine resulted in promoter demethylation and re-expression of *NKILA* transcript. In NHL primary samples (n=102), *NKILA* methylation was observed none of mantle cell lymphoma (MCL) cases, but in 29 (51.79%) diffuse large-B cell lymphoma (DLBCL) and 4 (20%) peripheral T-cell lymphoma (PTCL) cases, hence preferentially methylated in DLBCL than MCL ($P < 0.0001$) and PTCL ($P = 0.007$). Mechanistically, knockdown of *NKILA* resulted in promoting IkBα phosphorylation, which was associated with nucleus translocation of total p65 and phosphorylated p65 in SU-DHL-1 cells, hence constitutive NF-κB activation. Functionally, knock-down of *NKILA* in SU-DHL-1 cells led to decreased cell death and increased cellular proliferation, indicating a tumor suppressor role of *NKILA* in NHL cells.

Conclusions: *NKILA* was a tumour suppressor lncRNA frequently hypermethylated in DLBCL. Promoter DNA methylation-mediated *NKILA* silencing led to increase of cellular proliferation and decrease of cell death via repression of NF-κB signaling in NHL cells.

Background

Non-Hodgkin's lymphoma (NHL) encompasses a heterogeneous group of diseases, including B-cell, T-cell, and natural killer (NK)-cell lymphoma based on the origin and lineage of tumor cells [1]. B-cell NHL comprises more than 70% of all lymphoma while T-cell NHL accounts for 10–15% of all lymphoma [2]. Diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) are the most two common subtypes of NHL [3] whereas NK-cell lymphoma is an aggressive subtype rare in Western countries [4]. The incidence of NHL was 6.7/100,000 among males and 4.7/100,000 among females worldwide, which ranked the 8th and 10th of all types of cancers respectively [5]. The clinical presentations of NHL patients include painless lymphadenopathy, organomegaly and presence or absence of B symptoms (night sweats, weight loss > 10% and fever with temperature > 38 °C) [6].

DNA methylation refers to the addition of methyl group (-CH3) to C5 position of cytosine in a CpG dinucleotide via DNA methyltransferases [7]. Cancer cells are characterized by two major alterations of DNA methylation: global DNA hypomethylation but gene-specific DNA hypermethylation of promoter-associated CpG island [8]. Moreover, promoter DNA hypermethylation-mediated reversible silencing of

tumor suppressor protein-coding genes, including *PTPL1* [9] and *SHP1* [10, 11], and tumor suppressive miRNAs, including *miR-155-3p* [12] and *miR-146a* [13], has been implicated in lymphomagenesis.

Long non-coding RNA (lncRNA) is broadly defined as class of non-coding RNA measuring > 200 nucleotides [14, 15]. LncRNAs play essential roles in regulating multiple biological processes, including cellular metabolism, organogenesis and carcinogenesis [16–18]. LncRNA, *NKILA* (*NF-KappaB Interacting LncRNA*), localized to 20q13.31, was firstly found to be downregulated in breast cancer. Overexpression of *NKILA* resulted in inhibition of metastasis and increase of apoptosis by repression of NF-κB signaling activity in breast cancer cells, indicating the tumor suppressor property of *NKILA* [19]. Moreover, NF-κB signaling pathway is constitutively activated and hence implicated in the pathogenesis of NHL. However, the function of *NKILA* in lymphoma remains unknown.

As a CpG island is present at the promoter region of *NKILA*, we postulated that *NKILA* is a tumor suppressor lncRNA reversibly silenced by promoter DNA methylation in NHL. Herein, the methylation of *NKILA* will be studied in NHL, and the role of *NKILA* in lymphomagenesis will also be investigated.

Results

Methylation of NKILA in normal healthy controls and NHL cell lines

The methylation status of *NKILA* was investigated by MSP in the bisulfite-converted DNA of normal healthy controls, including 10 peripheral blood buffy coats and 11 normal tonsil tissues, in addition to 10 NHL cell lines. Direct sequencing of M-MSP products from methylated positive control DNA demonstrated that all unmethylated cytosines were converted into thymidines after PCR, whereas all methylated cytosines remained unchanged, indicating the complete bisulfite conversion and specificity of MSP (Fig. 1a). By MSP, methylation of *NKILA* was absent in all of normal peripheral blood buffy coats and normal tonsil tissues (Fig. 1b). Amongst NHL cell lines, *NKILA* was completely methylated (MM) in SU-DHL-6 and completely unmethylated (UU) in GRANTA-519, JEKO-1, MINO, REC-1, SP-53, KARPAS-299 and SU-DHL-1. However, neither U-MSP nor M-MSP signals were observed in SUP-T1 cells (Fig. 1c). Furthermore, the methylation status of *NKILA* in NHL cell lines detected by MSP was verified by quantitative bisulfite pyrosequencing. SU-DHL-6 cells with complete methylation of *NKILA* had a mean methylation percentage of 69.4%. In contrast, NHL cell lines with completely unmethylation of *NKILA* had a mean methylation percentage ranging from 5.0–6.5%, which confirmed the methylation status detected by MSP (Fig. 1d). These results indicated that *NKILA* was methylated in a tumor-specific manner in NHL cells.

Methylation and expression of NKILA in NHL cell lines

To explore the relationship between promoter DNA methylation and the expression of *NKILA*, semi-quantitative RT-PCR of *NKILA* was performed in NHL cell lines. As demonstrated by the DNA gel, no expression of *NKILA* was detected in SU-DHL-6 cells that was completed methylated for *NKILA.*

Conversely, expression of *NKILA* was observed in other cell lines completely unmethylated for *NKILA* (Fig. 2a).

Furthermore, to study whether promoter DNA methylation was associated with reversible silencing of *NKILA*, SU-DHL-6 cells, which were completely methylated for *NKILA*, were treated with a demethylating agent, 5-AzadC for 7 days. Upon treatment with 5-AzadC, the promoter of *NKILA* was demethylated as illustrated by the emergence of U-MSP signal (Fig. 2b), with re-expression of *NKILA* (Fig. 2c). Hence, these data suggested that reversible silencing of *NKILA* was mediated by promoter DNA methylation in NHL cells.

Methylation of NKILA in primary NHL samples

To investigate the methylation of *NKILA* in NHL primary samples, MSP was performed with bisulfite-converted DNA in primary samples, including 26 mantle cell lymphoma (MCL), 56 DLBCL and 20 peripheral T-cell lymphoma (PTCL). MSP results showed that no methylation of *NKILA* was detected in primary MCL samples. However, *NKILA* was found to be methylated in 29 (51.79%) DLBCL and 4 (20%) PTCL cases (Fig. 3a-c), hence preferentially methylated in DLBCL than MCL ($P < 0.0001$) and PTCL ($P = 0.007$).

NKILA inhibiting IkBα phosphorylation and NF-κB activation

NKILA has been reported to suppress NF-κB signaling pathway by blocking IkBα phosphorylation in breast cancer, non-small cell lung cancer and nasopharyngeal carcinoma [19–21]. To elucidate *NKILA* function in lymphoma, *NKILA*-targeted siRNA was used to knock down *NKILA* and non-targeting siRNA was used as a control in SU-DHL-1. The qRT-PCR result confirmed *NKILA* was knocked down at 24-hours and 48-hours post-transfection (Fig. 4a). To further evaluate *NKILA* function in NF-κB signaling pathway, we examined IkBα phosphorylation and p65 nucleus translocation after *NKILA* knock-down, with augmentation of the NF-κB signaling by TNFα [19]. The result showed that *NKILA* knock-down led to increase of IkBα phosphorylation in SU-DHL-1 at both 24-hours and 48-hours post-transfection (Fig. 4b), which was associated with enhanced p65 translocated into nucleus compared to the control. Moreover, enhanced nuclear translocation of phosphorylated p65 ser536 was observed after *NKILA* knock-down (Fig. 4c). These results collectively indicated *NKILA* negatively regulated NF-κB signaling pathway by inhibiting IkBα phosphorylation, and reduced nuclear translocation of total and phosphorylated p65.

Effect of knock-down of NKILA on SU-DHL-1 cells

As *NKILA* plays as a negative regulator in NF-κB signaling pathway, its tumor suppressor function in lymphoma was further explored by examining cell proliferation and cell death in SU-DHL-1 cells that were completely unmethylated for *NKILA*. Knock-down of *NKILA* led to a significantly increased cell proliferation rate compared to the control (Fig. 5a). Furthermore, knock-down of *NKILA* resulted in reduced cell death in SU-DHL-1 (Fig. 5b). These results supportively indicated *NKILA* acted as a tumor suppressor lncRNA in SU-DHL-1.

Discussion

Several observations were made in this study. Firstly, we showed that *NKILA* was methylated in NHL cell lines and NHL primary samples but unmethylated in normal controls, hence methylated in tumor-specific manner in NHL. This was consistent with tumor-specific pattern of methylation of tumor suppressor protein-coding genes, such as *p16* and *p15* [22], and non-coding tumor suppressor miRNAs, such as *miR-124-1* [23] and *miR-34a* [24] in NHL. However, this contrasted with the tissue- but not tumor-specific pattern of methylation, such as *miR-373* [25] and *miR-127* [26], were shown to be methylated in both normal counterparts and tumor cells, hence likely unimportant in carcinogenesis.

Secondly, in primary NHL samples, *NKILA* was frequently methylated in DLBCL but not MCL or PTCL samples. Indeed, NHL is highly heterogenous with different genetic and epigenetic features [31]. For instance, microarray-based DNA methylation study in 367 hematological neoplasms demonstrated that promoter DNA hypermethylation was more frequent in precursor B and T lymphoid neoplasias and mature B-cell lymphomas of germinal center origin (such as DLBCL, FL and Burkitt's lymphoma) than mature T-cell lymphomas such as PTCL [32]. Therefore, differential methylation of *NKILA* might be accounted by the difference in cell of origin and their inherent pathogenetic mechanisms.

Thirdly, to our knowledge, this is the first study that reported promoter DNA methylation mediated the reversible silencing of *NKILA* in NHL, which is evidenced by an inverse correlation between the *NKILA* methylation and its expression in NHL cell lines, and re-expression of *NKILA* upon demethylation treatment in cell line with complete methylation of *NKILA*. Apart from promoter DNA methylation, *NKILA* have been reported to be regulated by other mechanisms. For instance, Huang et al [27] showed that, in cytotoxic T lymphocytes, *NKILA* transcription induced by antigen stimulation was mediated by increase of the acetylation of histones (H4ac, H3K27ac, and H3K9ac) at the promoter region of *NKILA*, suggesting the expression of *NKILA* regulated by histone modification. In addition, in breast cancer cells, some oncogenic miRNAs, such as *miR-103* or *miR-107*, could directly target and downregulate the expression of *NKILA*, which was confirmed by luciferase reporter assay [19].

Fourthly, *NKILA* was first shown to suppress the NF-κB signaling in breast cancer. It could directly bind to NF-κB/IκB complex and mask the phosphorylation site of IκB, thereby suppressing IKK-induced IκB phosphorylation and hence NF-κB activity [19]. As constitutive NF-κB activation is the hallmark of many lymphoid malignancies, including Hodgkin lymphoma, DLBCL and multiple myeloma [28] and hence implicated in lymphomagenesis, it is essential to comprehend the precise function of *NKILA* and its interaction with NF-κB in lymphoma. To the best of our knowledge, this is the first study showing the role of *NKILA* in lymphoma. Previous studies reported that phosphorylation p65 at ser536 led to enhanced transcriptional activity of NF-κB [29–31]. We observed that knockdown of *NKILA* led to upregulation of NF-κB signaling pathway by promoting IκBα phosphorylation, and consequent nucleus translocation of total p65 and phosphorylated p65 in lymphoma cells. Furthermore, as a transcription factor, NF-κB regulates expression of multiple downstream effectors, enhancing cell proliferation, inhibiting cell apoptosis, or promoting cell migration and invasion [32]. Herein, in NHL cells, down-regulation of *NKILA*

resulted in increase of cellular proliferation and decrease of cell death, consistent with the tumor suppressor role of *NKILA* in multiple tumors, including melanoma, lung cancer, rectal cancer, laryngeal cancer and breast cancer [19, 20, 33–36]. In addition, *NKILA* has also been shown to inhibit tumor invasion and migration in epithelial cancers, such as breast cancer, hepatocellular carcinoma and tongue squamous cell carcinoma [19, 36, 37]. Collectively, these results suggested that the *NKILA* could inhibit cellular proliferation and induce cell death by suppressing the NF-κB signaling pathway in NHL cells.

Conclusions

In NHL, epigenetic silencing of lncRNA *NKIL*A was mediated by promoter DNA methylation in a tumor-specific manner. Frequent hypermethylation of *NKILA* was preferentially detected in DLBCL. *NKILA* exerted its tumor suppressive property by inhibition of cellular proliferation and increase of cell death in NHL cells in association with suppression of NF-κB signaling pathway.

Materials And Methods

Patient samples

One hundred and two formalin fixed, paraffin-embedded (FFPE) or fresh frozen diagnostic lymph node biopsy tissues, including 56 DLBCL, and 26 MCL and 20 PTCL cases, were acquired from five hospitals in Hong Kong (Queen Mary Hospital, Kwong Wah Hospital, Princess Margaret Hospital, United Christian Hospital and Pamela Youde Nethersole Eastern Hospital). The diagnosis of lymphoma was based on the WHO (World Health Organization) classification [38]. Eleven FFPE tonsil tissues were also obtained from healthy individuals undergoing tonsillectomy. Our study was approved by the Institutional Review Board of Queen Mary Hospital.

Cell culture

Five MCL cell lines (SP53, REC-1, GRANTA-519, MINO and JEKO-1), two DLBCL cell lines (SU-DHL-6 and SU-DHL-16), two ALK (+) anaplastic large cell lymphoma (ALCL) cell lines (KARPAS-299 and SU-DHL-1) and one T-cell lymphoblastic lymphoma cell line (SUP-T1) were used in this study. SP53 and REC-1 were kindly provided by Prof Raymond Lai (Department of Laboratory Medicine and Pathology, University of Alberta and Cross Cancer Institute). Other cell lines were purchased from Deutsche Sammlung von Mikroogranismen und Zellkulturen (DSMZ) (Braunschweig, Germany). Cell lines were maintained in RPMI-1640 (DMEM for GRANTA-519) supplemented with 10-15% fetal bovine serum, 50U/mL of penicillin and 50ug/ mL streptomycin in a humidified atmosphere of 5% CO2 at 37°C.

DNA demethylation treatment

SU-DHL-6 cells (1×10^6 cells/ml) were seeded in 25 cm^2 flasks and cultured with 0.5~1.5 μM of 5-aza-2'-deoxycytidine (5-azadC) (Sigma–Aldrich) for seven days. 5-azadC was replaced at every 24 hours. Afterwards, the cells were harvested for DNA and RNA extraction.

DNA and RNA extraction

DNA from NHL cell lines and healthy peripheral blood was extracted with DNA Blood Mini kit (Qiagen). DNA extraction from frozen patient biopsies was conducted with automated DNA extraction system (DNA Tissue Kit from Qiagen). DNA extraction from FFPE tissues performed by using QIAamp DNA FFPE Tissue Kit (Qiagen). Total RNA was extracted with Direct-zol™ RNA MiniPrep kit (Zymo Research).

Methylation-specific polymerase chain reaction (MSP)

Sodium bisulfite conversion was conducted with EpiTect Bisulfite Kit (Qiagen). Afterwards, MSP was performed in bisulfite-treated DNA with two sets of primers, which were specific to unmethylated (U-MSP) or methylated (M-MSP) DNA sequence. MSP primers were designed at the CpG island upstream to *NKILA* gene by online tool Methprimer (http://www.urogene.org/methprimer/). Details of primer sequence and PCR condition for MSP were listed in Table 1. For each MSP reaction, the enzymatically methylated control DNA (CpGenome Universal Methylated DNA; Chemicon / Millipore, Billerica, MA, USA) was used as positive control for M-MSP and negative control for U-MSP.

Table 1
Primer sequences and PCR reaction conditions for *NKILA*

	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Tm/cycles/$MgCl_2$	Reference
Methylation-specific PCR (MSP)				
M-MSP	TAG GTA GAC GGT TTG ACG TTA GC	GAA AAA ACC TCG ACG AAA ATT AAC G	57°C/35x/2 mM	
U-MSP	GGT AGG TAG ATG GTT TGA TGT TAG T	ACA AAA AAA CCT CAA CAA AAA TTA ACA	55°C/37x/1.5 mM	
Semi-quantitative RT-PCR/ Quantitative real-time RT-PCR				
NKILA	AAC CAA ACC TAC CCA CAA CG	ACC ACT AAG TCA ATC CCA GGT G	55°C/40x/2 mM (Semi-quantitative RT-PCR)	[19]
GAPDH	ACC ACA GTC CAT GCC ATC ACT	TCC ACC ACC CTG TTG CTG TA	60°C/24x/1.5 mM (Semi-quantitative RT-PCR)	[24]
Key: M-MSP: MSP for methylated alleles; U-MSP: MSP for unmethylated alleles; Tm: annealing temperature				

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated with the mirVanaTM miRNA Isolation Kit (Invitrogen), followed by reverse transcription with SuperScript® III (Invitrogen). qRT-PCR was performed with SYBR® Select Master Mix

(ABI), and the human glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the endogenous control. The relative quantity of *NKILA* expression was calculated by the method of 2^{-DDCt} and normalized against the endogenous control. The primer sequences for *NKILA* and *GAPDH* were listed in Table 1 [19, 24].

Quantitative bisulfite pyrosequencing

The promoter region of *NKILA* overlapped with the amplicon of MSP was amplified in the bisulfite converted DNA with methylation-unbiased primers. Primer sequences and condition for PCR were listed as follows: forward primer: 5'-GTT GGG GAG AGG GTA TAG-3', reverse primer: 5'-Biotin- CTC CTC CTC CTC ATT CAA ATC -3'; condition: 56℃/45x/1.5mM. Qiagen PyroMark PCR Kit was employed to perform PCR amplification. Afterwards a stretch of DNA containing 10 consecutive CpG dinucleotides were pyrosequenced with sequencing primer: 5'-GTT AGG GGA GGG GGT G-3', on a PSQ 96MA system and analyzed by PyroQ-CpG 1.0.9. software.

Knockdown of *NKILA*

RNA interference by small interfering RNA (siRNA) was used to knockdown the expression of *NKILA*. Briefly, SU-DHL-1 cells were seeded at a density of 0.5×10^6/ml in a six-well plate and transfected with *NKILA* siRNA (n541256, Ambion, USA*)* or Silencer Negative Control (Ambion, USA) at final concentration of 150 nM with RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA, USA). The transfected cells were cultured for 48 hours.

Cell proliferation and cell death

After 24-hours and 48-hours transfection, viable cells were analyzed by trypan blue exclusion and cell number was measured by the Countess II (Invitrogen). Cell proliferation was calculated by normalization with the negative control at 48-hours post-transfection. Cell death was analyzed by trypan blue exclusion at 24-hours post-transfection. All experiments were repeated at least three times.

Western blot

SU-DHL-1 cells transfected with *NKILA* siRNA and negative control were treated with 200ng/ml TNFα for one hour before lysed in RIPA buffer (Cell signaling). Totally, 15μg protein of each sample was loaded and separated in Mini-PROTEAN TGXTM Precast gel (Bio-rad), followed by transferred onto a 0.45 μm PVDF membrane (Amersham). The membrane was blocked in Super blocking buffer and incubated in primary antibodies including p65 (1:1000, Cell signaling), p-p65 ser536 (1:1000, Cell signaling), IkBα (1:1000, Cell signaling), p-IkBα ser32 (1:1000, Cell signaling), β-tubulin (1:1000, Cell signaling), nucleolin (1:1000, Cell signaling) at four degree overnight with gently rotation. The membrane was then washed and incubated with HRP-linked secondary antibodies, anti-mouse (1:3000, Cell signaling) and anti-rabbit (1:3000, Cell signaling) for one hour, followed by TBST washing. The ECL HARP substrate was used on the membrane before developed in X-ray films.

Nuclear and cytosol fractionations of SU-DHL-1 cells were prepared by the Nuclear/Cytosol Fractionation Kit (BioVision), according to the manufacturers' instructions. The samples were then performed Western blot.

Statistical Analysis

The difference of cell proliferation and cell death between SU-DHL-1 cells transfected with *NKILA* siRNA and Silencer Negative Control were compared by Student's t-test. The difference of *NKILA* methylation frequency in different subtypes of NHL primary samples was analyzed by χ^2 test. All P-values were 2-sided. $P < 0.05$ was considered as significant difference.

Declarations

Ethics approval and consent to participate

The study has been approved by the Institutional Review Board of Queen Mary Hospital (UW 05-269 T/932), and written informed consent has been obtained from patients for the participation of this study.

Consent for publication

All the co-authors consent to publish the work in Cell & Bioscience.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions:

CS Chim and MY Zhang conceived and designed the study. MY Zhang MD Deng and LQ Wang carried out the experiments. CS Chim and MY Zhang drafted the manuscript. RKH Au-Yeung conducted pathology review of the lymphoma cases. All authors read and approved the final manuscript.

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Figures

Figure 1

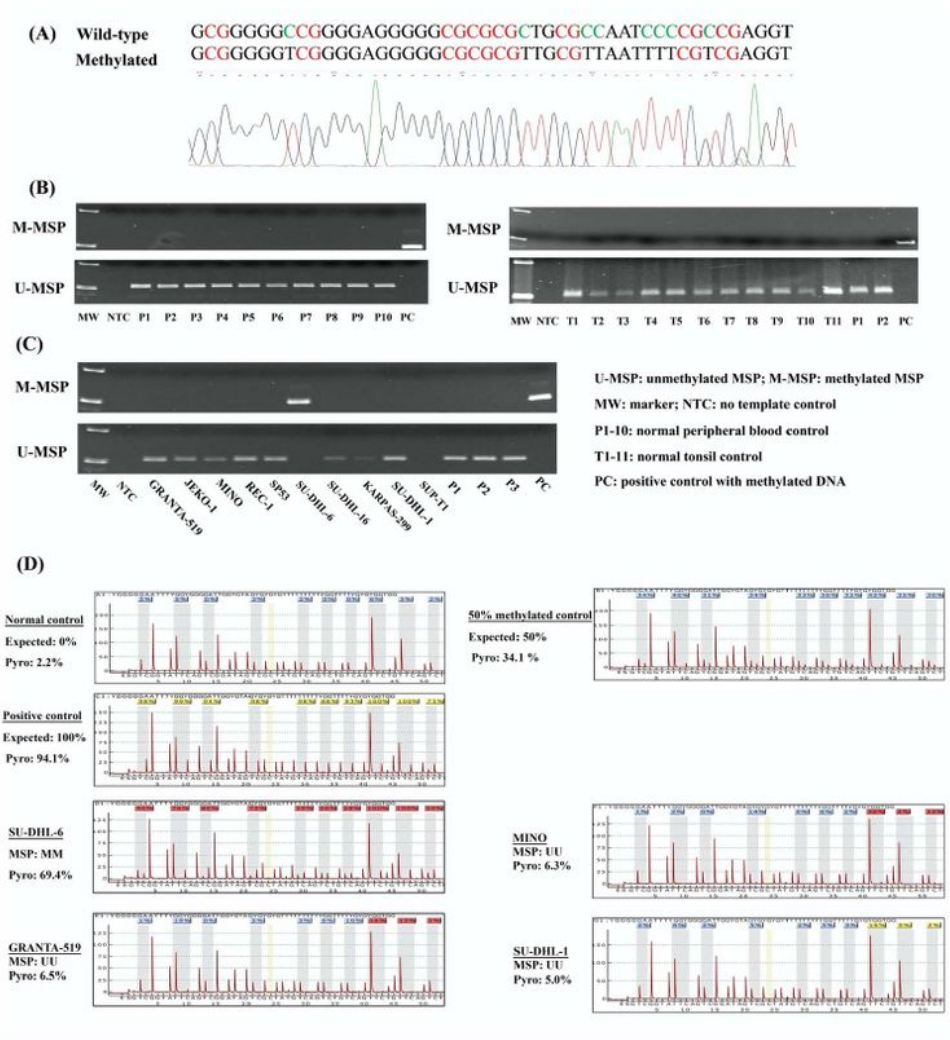

Figure 1

Methylation of NKILA in normal controls and NHL cell lines. a Direct sequencing of M-MSP products from positive control with methylated DNA. b M- and U-MSP demonstrated that NKILA was unmethylated in normal peripheral blood buffy coats (P1-P10) and normal tonsil tissues (T1-T11). c M- and U-MSP showed that NKILA was completely methylated in SU-DHL-6 and completely unmethylated in GRANTA-519, JEKO-1, MINO, REC-1, SP-53, KARPAS-299 and SU-DHL-1. Both M-MSP and U-MSP was absent in

SUP-T1 cells. d Quantitative bisulfite pyrosequencing analysis showed mean methylation percentage of 10 neighboring CpG dinucleotides overlapped MSP amplicon in 0%, 50% and 100% methylation control, and NHL cell lines.

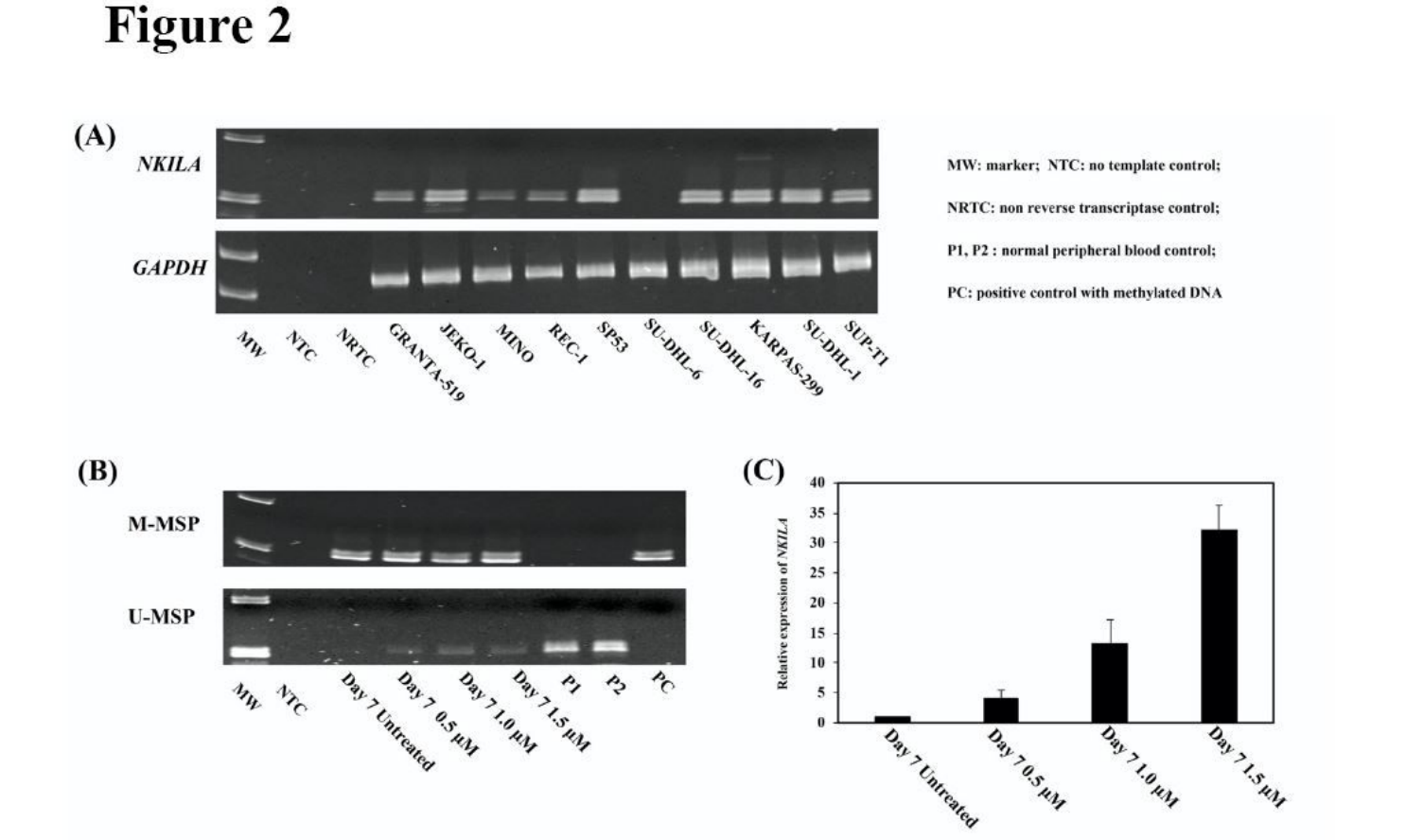

Figure 2

Correlation of DNA methylation of NKILA and its expression in NHL cell lines. a NKILA methylation measured by MSP was associated with lower expression of HOTTIP in NHL cell lines by semi-quantitative RT-PCR. b, c Treatment of SU-DHL-6 cells completely methylated for NKILA with 5-AzadC for 7 days led to NKILA promoter demethylation detected by MSP (b), and associated with re-expression of NKILA by qRT-PCR (c). Columns represented mean +/- 1SD from three qRT-PCR experiments in triplicate.

Figure 3

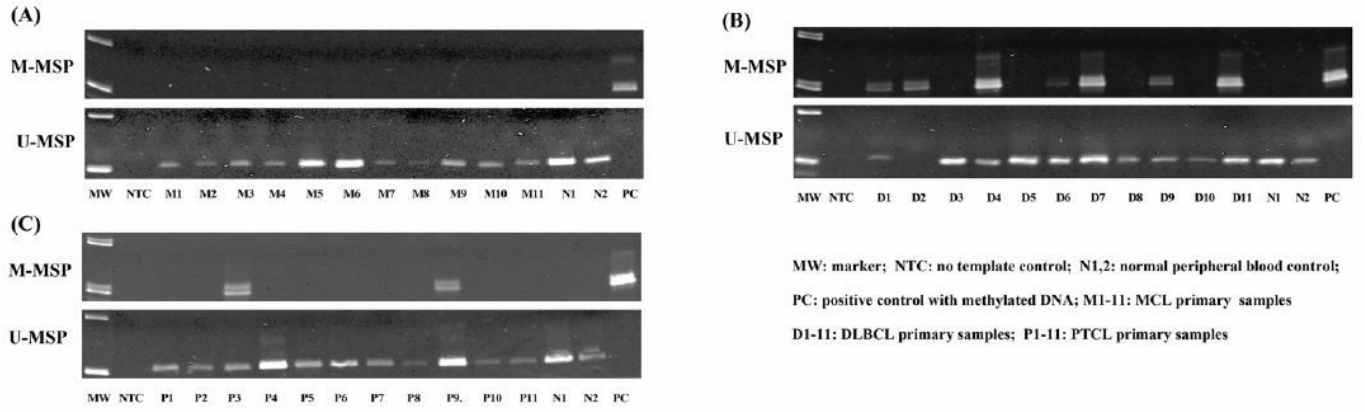

Figure 3

Methylation of NKILA in primary NHL samples. Representative M- and U-MSP showed methylation of NKILA in MCL (a), DLBCL (b) and PTCL (c).

Figure 4

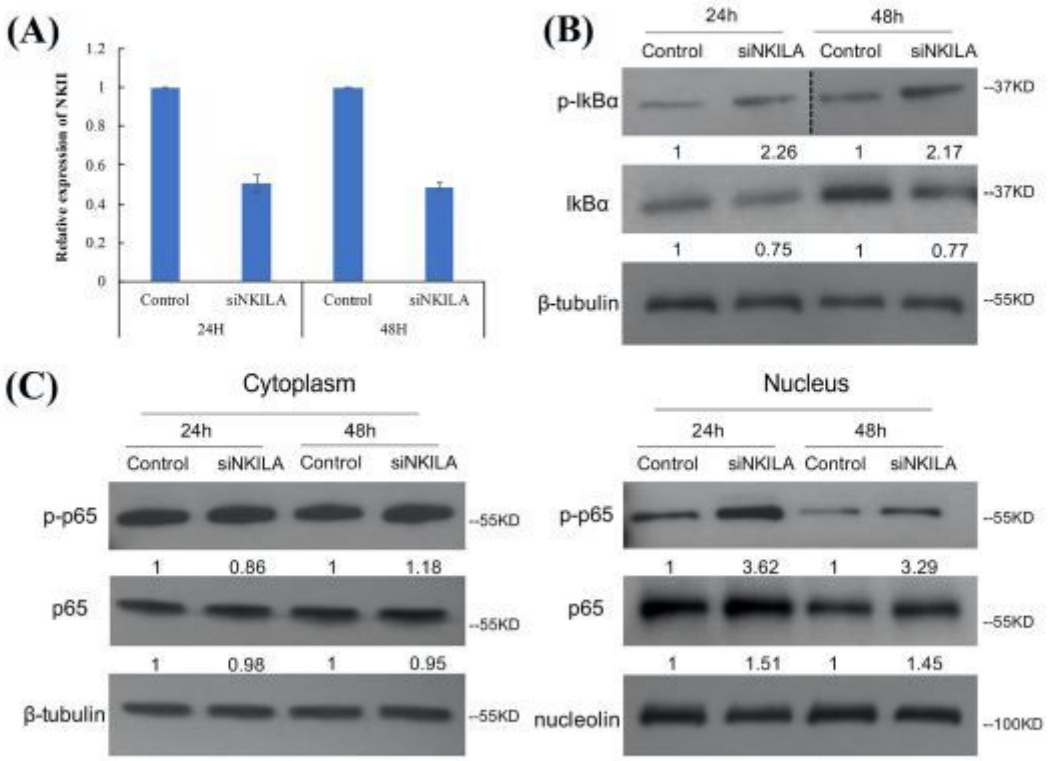

Figure 4

NKILA knock-down led to more IκBα phosphorylation and more p65 translocated into nucleus. a qRT-PCR was performed to examine NKILA knock-down efficiency in SU-DHL-1 at 24-hour and 48-hour post-transfection. Columns represented mean +/- 1SD from three independent experiments. b Western blot shows total IκBα and phosphorylated IκBα ser32 expression level. β-tubulin was used as the loading control. Quantification densitometry of each band is normalized with the loading control. p-IκBα: phosphorylated IκBα ser32. c Western blot shows total p65 and phosphorylated p65 ser536 level in cytoplasm and nucleus. β-tubulin and nucleolin serves as the loading control for cytoplasm and nucleus, respectively. p-p65: phosphorylated p65 ser536; siNKILA: NKILA-targeted siRNA. 24h: 24-hours post-transfection; 48h: 48-hours post-transfection.

Figure 5

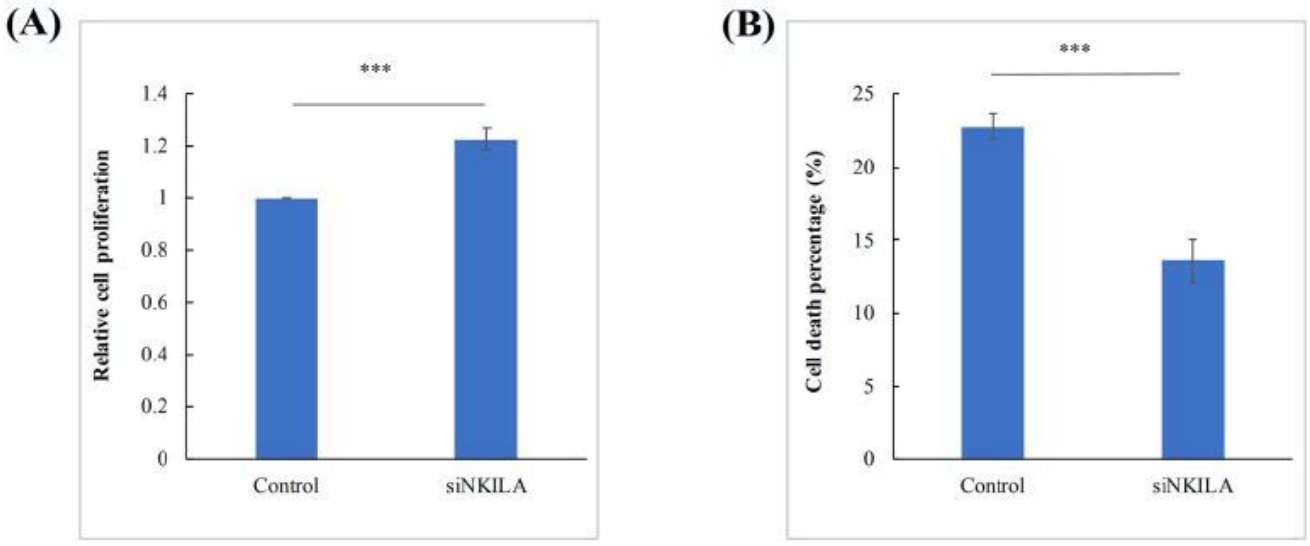

Figure 5

NKILA suppressed cell growth and promoted cell death in SU-DHL-1. a Cellular proliferation upon knockdown of NKILA was studied by trypan blue assay at 48-hours post-transfection. Columns represented mean +/- 1SD from five independent experiments. b Cell death upon knockdown of NKILA was studied by trypan blue exclusion assay at 24-hours post-transfection. Columns represented mean +/- 1SD from three independent experiments. siNKILA: NKILA-targeted siRNA. ***: P-value 0.001