Archives of Dermatological Research (2025) 317:559
https://doi.org/10.1007/s00403-025-03964-y

ORIGINAL PAPER

Overexpression of lncRNA TINCR inhibits cutaneous squamous cell carcinoma cells through promotes methylation of Myc and TERC genes

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Received: 27 November 2024 / Revised: 31 December 2024 / Accepted: 3 February 2025

Abstract

Long non-coding RNA (lncRNA) TINCR has been shown to play a crucial regulatory role in various tumors. However, its specific mechanism of action in cutaneous squamous cell carcinoma (CSCC) remains unclear. This study aimed to explore the role of lncRNA TINCR in CSCC. We utilized overexpression techniques to study the effects of TINCR on CSCC cells. Methylation-specific PCR (MSP) and RNA immunoprecipitation (RIP) assays were used to assess the impact of TINCR on the methylation of the promoter regions of the Myc and TERC genes, and its interaction with DNA methyltransferase 1 (DNMT1). The results showed that overexpression of TINCR significantly promoted methylation in the promoter regions of Myc (N-MYC, L-MYC, and c-MYC) and TERC genes, inhibiting the proliferation, migration, and invasion of CSCC cells. MSP and RIP experiments further confirmed that TINCR binds to DNMT1, enhancing the methylation levels of the promoter regions of Myc and TERC genes. These findings suggest that lncRNA TINCR may serve as a potential therapeutic target for CSCC by regulating the methylation of key oncogenes. These findings provide new insights into the molecular mechanisms of CSCC and highlight the therapeutic potential of targeting TINCR.

Keywords Long non-coding RNA · Methylation · Cutaneous squamous cell carcinoma · Cell proliferation · Cell migration

Introduction

CSCC is the second most common skin cancer worldwide, particularly prevalent among elderly individuals with prolonged sun exposure. Although the external factors influencing CSCC, such as ultraviolet (UV) radiation, have been extensively studied, its molecular mechanisms remain poorly understood. The development of CSCC involves multiple pathophysiological processes, including genetic mutations, cell cycle dysregulation, changes in the immune microenvironment, and abnormal epigenetic modifications [1].

UV radiation is the primary external cause of CSCC and can directly cause DNA damage and trigger genetic mutations. Mutations in the TP53 gene are particularly common in CSCC, leading to a failure in the cellular response to DNA damage, which results in continuous cell proliferation [2]. Additionally, DNA methylation, a key epigenetic modification, plays a critical role in the development of CSCC [3]. Studies have shown that certain tumor suppressor genes, such as CDKN2A, are silenced in CSCC cells due to hypermethylation, further promoting tumor growth and invasion [4]. The degradation and remodeling of the extracellular matrix (ECM) is another crucial factor that enhances the invasiveness and metastatic potential of CSCC. Matrix metalloproteinases (MMPs) secreted by tumor cells degrade the surrounding ECM, providing a pathway for tumor invasion. Moreover, tumor-associated inflammatory responses promote angiogenesis and immune evasion, further exacerbating CSCC progression [5].

lncRNA TINCR is a critical non-coding RNA molecule discovered in recent years. It is believed to play a key role

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Published online: 12 March 2025

in epidermal cell differentiation by interacting with various RNA-binding proteins to regulate mRNA stability and translation efficiency [6]. Studies have shown that TINCR is involved in multiple physiological processes through the regulation of downstream gene transcription, RNA processing, translation, and protein modification, particularly in cell proliferation, differentiation, and tumorigenesis [7]. Anli Hou and colleagues found that lncRNA TINCR sponges miR-302 to upregulate cyclin D1 in cervical squamous cell carcinoma [8], while Wu Zhou and colleagues discovered that lncRNA TINCR is involved in ALA-PDT-induced apoptosis and autophagy in CSCC [9]. Additionally, in colorectal cancer research, it was found that lncRNA DACOR1 can recruit DNA methyltransferase DNMT1 to regulate DNA methylation [10]. The critical role of lncRNAs in regulating DNA methylation in human cells, and the potential dysregulation of these lncRNAs, may represent a key mechanism in altering DNA methylation patterns in human tumors.

Myc and TERC represent proto-oncogenes and the telomerase RNA component, respectively, each playing distinct roles in the organism and closely associated with the development and progression of various diseases [11]. Myc is a vital oncogene in many cancers, and its overexpression leads to abnormal cell proliferation and malignant transformation. By regulating the transcription of downstream target genes, Myc promotes cell cycle progression and inhibits apoptosis, thereby driving tumor development and progression [12]. The methylation regulation of the Myc gene may reduce its expression, inhibiting the malignant behavior of CSCC cells. TERC is a crucial component of telomerase, which is responsible for maintaining telomere length and preventing chromosome end loss. TERC forms a complex with telomerase reverse transcriptase (TERT) to elongate telomeres during cell division, maintaining the replicative potential of cells. In cancers such as CSCC, the overexpression of TERC can activate telomerase, allowing cancer cells to gain unlimited proliferative ability, thereby promoting tumor progression [13]. The aberrant expression of both Myc and TERC is closely related to the malignant behavior of cancer cells. It is hypothesized that lncRNA TINCR, by recruiting DNMT1 to methylate the promoter regions of these genes, may effectively suppress their expression, thereby reducing the proliferation, migration, and invasion capacity of CSCC cells.

Currently, research on TINCR in CSCC is gradually increasing. Existing studies have shown that TINCR not only regulates gene expression through DNA methylation pathways but may also influence tumor cell behavior by interacting with other epigenetic factors and signaling pathways, such as Wnt/β-catenin and PI3K/AKT [14, 15]. Although these studies have provided us with a preliminary understanding of TINCR in CSCC, its specific molecular mechanisms remain to be further explored. Investigating the interactions between TINCR and epigenetic factors, exploring the relationship between TINCR and other cancer-related signaling pathways, and evaluating the potential of TINCR as a therapeutic target are all areas worthy of further study.

In this study, we used a series of cellular and molecular biology techniques to explore the function of lncRNA TINCR in CSCC. First, overexpression and knockdown vectors of lncRNA TINCR were constructed and transfected into the CSCC cell line A431, with RT-qPCR used to detect expression levels to assess the effects on cell proliferation, migration, and invasion. Subsequently, a CCK-8 assay was employed to evaluate cell proliferation, and a Transwell assay was used to assess cell migration and invasion. Additionally, to investigate the regulatory effects of TINCR on the methylation of Myc and TERC genes, RIP experiments were performed to detect the interaction between TINCR and DNMT1, and MSP was used to assess the methylation levels of the Myc and TERC gene promoter regions. These experimental results will provide new insights into the mechanism by which overexpression of lncRNA TINCR in CSCC cells interacts with DNMT1 to promote the methylation of Myc and TERC genes, leading to decreased expression of Myc and TERC and inhibition of cell proliferation, migration, and invasion in CSCC.

Materials and methods

Cell culture and transfection

Cell culture

The A431 skin cancer cell line, A375 melanoma cells, and HACAT cells used in this study were purchased from Xiamen Yimo Biotechnology Co., Ltd. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in an incubator at 37 °C with 5% CO^2. When the cells reached approximately 80% confluence, they were used for subsequent experiments.

Lentiviral vector construction and packaging

The lentiviral overexpression vector pLV-Puro-OE-TINCR was constructed by inserting the TINCR gene sequence into the pLV-Puro vector, synthesized by GenePharma (Suzhou, China). Lentiviral packaging was performed using the ViraPower Lentiviral Packaging System (Invitrogen) according to the manufacturer's instructions. Specifically, 2.25 μg of the pLV-Puro-OE-TINCR plasmid, packaging

plasmid mixture, and P3000 reagent were mixed with Lipofectamine 3000 reagent, diluted in Opti-MEM medium, and incubated at room temperature for 15 min. The mixture was then transfected into 293T cells at 70–80% confluence. After 8 h of transfection, the medium was replaced with fresh DMEM complete medium, and cells were further cultured for 48 h. The viral supernatant was collected, filtered through a 0.45 μm filter, and the viral titer was determined before storing the viral solution at -80 °C.

Cell transfection

The pre-prepared lentivirus pLV-Puro-OE-TINCR or control virus was added to A431 cells cultured to 80% confluence, with polybrene (final concentration of 8 μg/mL) added to each well to enhance viral infection efficiency. After 8 h of infection, the virus-containing medium was removed and replaced with fresh DMEM complete medium containing 10% FBS, and the cells were further cultured for 24 h. Subsequently, the medium was replaced with a selective medium containing 1 μg/mL puromycin, and the cells were continuously selected for 7 days to obtain a stable TINCR-overexpressing cell line. The expression level of TINCR was verified by RT-qPCR to confirm the transfection efficiency.

Knockdown experiment

The knockdown experiment utilized a lentiviral vector pLKO.1 carrying TINCR shRNA, also synthesized by GenePharma (Suzhou, China). The constructed shRNA plasmids included three pairs of shRNA sequences targeting TINCR and a negative control (sh-NC). The transfection process was similar to that used in the overexpression experiment, with puromycin-containing medium used for selection after transfection, and the knockdown efficiency was verified by RT-qPCR.

Table 1 The primer sequences

Gene	Primer sequence(5'–3')
TINCR	Forward: GAAGCGCTACCACATCAAGG
	Reverse: CACCGTCTGGTGGTCGTC
N-MYC	Forward: GGAACTGTGTTGGAGCCGA
	Reverse: TTTGCAAGCCCTGCTCCTTAC
L-MYC	Forward: TGGAGCGAGGTGAGGCT
	Reverse: GGCAGCGAGTTCAAAGCAAA
c-MYC	Forward: TTCATAACGCGCTCTCCAAGTA
	Reverse: AGAGCGTGGGATGTTAGTGT
GAPDH	Forward: GAAAGCCTGCCGGTGACTAA
	Reverse: GCATCACCCGGAGGAGAAAT

RT-qPCR analysis

A431 cells were cultured under standard conditions and lysed with Buffer RL to extract RNA. The lysate was processed using FastPure gDNA-Filer Columns III and anhydrous ethanol, followed by washing with Buffer RW1 and RW2. The RNA was eluted with RNase-free ddH_2O, and its concentration and purity were measured using a Nano 600 spectrophotometer. Next, 500 ng of RNA was used as a template for reverse transcription with the Hiscript III RT SuperMix for qPCR kit to generate cDNA, which was stored at -20 °C. For real-time quantitative PCR, the cDNA was diluted and mixed with SYBR qPCR Master Mix and specific primers, and amplification was performed using a CFX96 Touch real-time PCR system. The PCR program was set at 95 °C for 30 s of initial denaturation, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. A melt curve analysis was performed at the end to confirm the specificity of the amplification. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown as Table 1.

CCK-8 cell proliferation assay

Logarithmically growing A431 cells were seeded in a 96-well plate at a density of 2,000 cells per well and incubated at 37 °C with 5% CO^2 for 6 h to ensure proper cell attachment. Each experimental group included six replicates to ensure data reliability. After 24 h of incubation, 10 μL of CCK-8 solution was added to each well 2 hours before the end of the incubation period. The CCK-8 solution reacts with intracellular reductase to produce a yellow formazan compound, whose absorbance is proportional to the number of cells. The optical density (OD450) of each well was then measured at 450 nm using a microplate reader to assess and compare the proliferation ability of cells in different treatment groups.

Transwell invasion and migration assay

A 50 μL Matrigel dilution was prepared by mixing VMatrigel with RPMI-1640 medium at a 1:4 volume ratio and gently applied to the Transwell chamber, then incubated at 37 °C for 6 h to solidify. Subsequently, logarithmically growing cells were resuspended in serum-free medium, and the cell density was adjusted to 2×10^5 cells/mL. A 100 μL cell suspension was added to the upper chamber, while 600 μL of medium containing 10% fetal bovine serum was added to the lower chamber, ensuring that the insert was placed stably without air bubbles. The same concentration of drugs was added to both chambers, and the cells were cultured at 37 °C for 24 h. After the experiment, non-invading cells on

the upper side of the membrane were removed using a cotton swab, and the upper chamber was fixed with 4% paraformaldehyde for 20 min. The fixative was removed, and the cells were stained with 0.5% crystal violet for 10 min, followed by washing and drying of the chamber. The invaded cells were observed under a microscope, photographed, and the invasion rate was calculated.

Western blot analysis

Logarithmically growing A431 cells were seeded in a 6-well plate at a density of 1×10^6 cells per well and cultured at 37 °C with 5% CO^2 for 24 h. The cells were lysed using RIPA buffer containing PMSF, and the lysate was collected by centrifugation at 4 °C. The supernatant was stored at -80 °C. Protein concentration was determined using the BCA method: BCA working solution was prepared, a standard curve was established, and absorbance was measured after incubation at 37 °C. Proteins were separated by SDS-PAGE and transferred to a PVDF membrane. The membrane was blocked and sequentially incubated with primary and secondary antibodies, followed by washing with TBST. Finally, chemiluminescence reagent was used for detection, and protein expression was analyzed using Image J software. N-Myc Antibody #9405, L-Myc (E3M5P) Rabbit mAb #76,266, c-Myc Antibody #9402, β-Actin Antibody #4967 (Cell Signaling Technology, USA, dilution ratio 1:1000), Goat Anti-Rabbit IgG H&L/HRP (Bioss, China, dilution ratio 1:20000).

Table 2 The primer sequences

Gene	
N-MYC	Left M primer TTTAGAGTG TAGTCGGTGTAAGTTC
	Left M primer ATTTCCAT AAAAATCAAAAAAAACG
	Left U primer TTTTAGAGT GTAGTTGGTGTAAGTTTG
	Right U primer TTTCCATA AAAATCAAAAAAAACAC
L-MYC	Left M primer TAGTTCGG AGTGGGTAAGGAGTAC
	Right M primer CAAATAAA TTACACGCTAAAAAACG
	Left U primer TTAGTTTGG AGTGGGTAAGGAGTAT
	Right U primer AATAAATT ACACACTAAAAAACACA
c-MYC	Left M primer AAATTAGTT TATAAGGTTTTTGCGG
	Right M primer ATAAAAAA AATTTACCTAACACGTA
	Left U primer AAATTAGTT TATAAGGTTTTTGTGG
	Right U primer ATAAAAAA AATTTACCTAACACATA
TERC	Left M primer AGTTATTTA GGAGGTTGAGATACGA
	Right M primer TTAAAACG AAATCTTACTCTTTCGC
	Left U primer TAGTTATTT AGGAGGTTGAGATATGA
	Right U primer TAAAACAA AATCTTACTCTTTCACC

Methylation-specific PCR

The extracted total DNA was first modified for methylation. The DNA samples were mixed with M-Dilution Buffer and incubated at 42 °C for 30 min. Then, the CT Conversion Reagent was added, and the reaction was incubated in the dark at 80 °C for 60 min. The mixture was transferred to spin columns and subjected to multiple washing and centrifugation steps, followed by elution of the purified DNA with M-Elution Buffer. MSP was performed using specific primers (Table 2) and 2× KeyPo Master Mix. The PCR program included an initial denaturation at 95 °C for 5 min, followed by 36 cycles of 95 °C for 10 s and 60 °C for 35 s, and the specificity of the product was confirmed by melt curve analysis. The PCR products were analyzed by 2% agarose gel electrophoresis containing EB, and imaging was performed under UV light to assess the methylation status of the samples. The entire process, from DNA modification to PCR amplification and gel electrophoresis analysis, was conducted under strict conditions to ensure the reliability and reproducibility of the experimental results.

RNA immunoprecipitation assay

RIP assay was used to explore the interaction between proteins and RNA. First, 1×10^7 A431 cells were lysed in Buffer A containing 1% protease inhibitor and 1% RNase inhibitor, followed by centrifugation at 4 °C. The supernatant was transferred to a new EP tube containing spin columns. After multiple washes with Buffer A and Buffer D, the samples were incubated with specific antibodies at 4 °C overnight to allow binding between the antibodies and RNA. The next day, the beads were washed with Buffer A to remove non-specific bindings, and the bound RNA was collected using a magnetic rack. The eluted RNA was further washed with ethanol-containing wash buffer and purified using RC Columns. Finally, the RNA was eluted with RNase-free water and stored at -80 °C. The RNA was then reverse transcribed into cDNA using a reverse transcription kit using specific primers (Table 3), followed by quantification using SYBR Green real-time PCR.

Table 3 The primer sequences

Gene		5'→3'
LncRNA TINCR	F	TGTGGCCCAAACTCAGGGATACAT
	R	AGATGACAGTGGCTGGAGTTGTCA

Data analysis

Data analysis and graphing were performed using GraphPad Prism 9 (Version 9.4.0). All data are presented as means ± SD, and statistical differences between groups were analyzed using the T-test or one-way ANOVA. A p-value of less than 0.05 was considered statistically significant.

Results

Overexpression of TINCR significantly enhances the proliferative capacity of CSCC cells

Inhibiting the proliferation of cancer cells is a crucial strategy in cancer therapy. Under normal culture conditions, the expression level of lncRNA TINCR in normal epidermal cells (HACAT) was significantly higher than in the A431 skin cancer cell line and A375 melanoma cell line (Fig. 1A), suggesting that TINCR may play an important role in maintaining normal skin cell function. Its significant downregulation in cancer cells may be associated with its potential tumor-suppressive function. In A431 skin squamous cell carcinoma cells, overexpression of TINCR (EX-TINCR group) significantly increased TINCR expression, while knockdown of TINCR (sh-TINCR group) significantly decreased its expression (Fig. 1B). This result validates the effectiveness of the gene transfection method for regulating TINCR expression and lays the foundation for further investigation of TINCR's specific function in skin squamous cell carcinoma. Additionally, CCK-8 assay results showed that overexpression of TINCR significantly enhanced cell proliferation in A431 cells, while knockdown of TINCR significantly reduced proliferation (Fig. 1C). These results indicate that TINCR expression significantly affects A431 cell proliferation, suggesting that TINCR may act as a molecular factor promoting cell proliferation in the initiation and progression of skin squamous cell carcinoma.

Overexpression of TINCR significantly enhances the migration and invasion abilities of CSCC cells

The Transwell assay results showed that A431 cells EX-TINCR group exhibited significantly enhanced migration and invasion abilities (compared to the EX-NC group, $P<0.01$), whereas A431 cells with TINCR knockdown demonstrated significantly reduced migration and invasion abilities (compared to the sh-NC group, $P<0.01$) (Fig. 2A). This indicates that the expression level of lncRNA TINCR is positively correlated with the migration and invasion abilities of A431 cells. High expression of lncRNA TINCR in A431 cutaneous squamous cell carcinoma cells can significantly promote cell migration and invasion, while low expression has the opposite effect. These findings suggest that lncRNA TINCR may play a promotive role in the

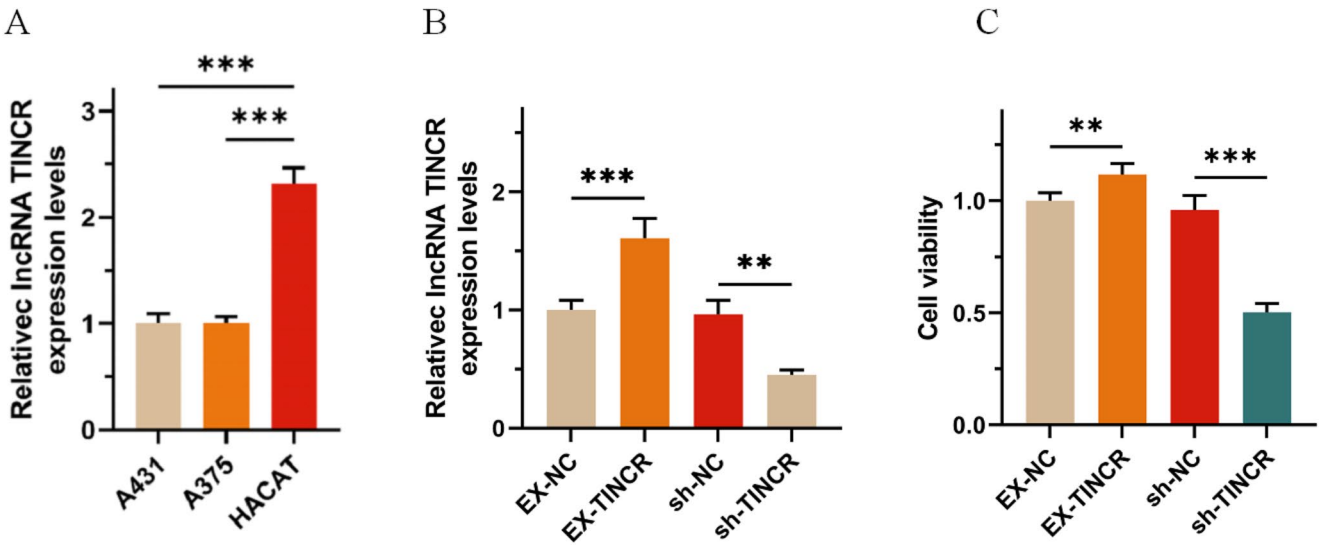

Fig. 1 Overexpression of TINCR significantly enhances the proliferative capacity of CSCC cells. (**A**) The expression level of lncRNA TINCR is significantly increased in the HACAT group compared to the A431 and A375 groups. (**B**) The expression level of lncRNA TINCR is significantly increased in the EX-TINCR group compared to the EX-NC group, while it is significantly decreased in the sh-TINCR group compared to the sh-NC group. (**C**) Cell proliferation is significantly increased in the EX-TINCR group compared to the EX-NC group, and significantly decreased in the sh-TINCR group compared to the sh-NC group. Experiments were repeated three times, and data are presented as mean ± standard error of the mean (SEM). *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$

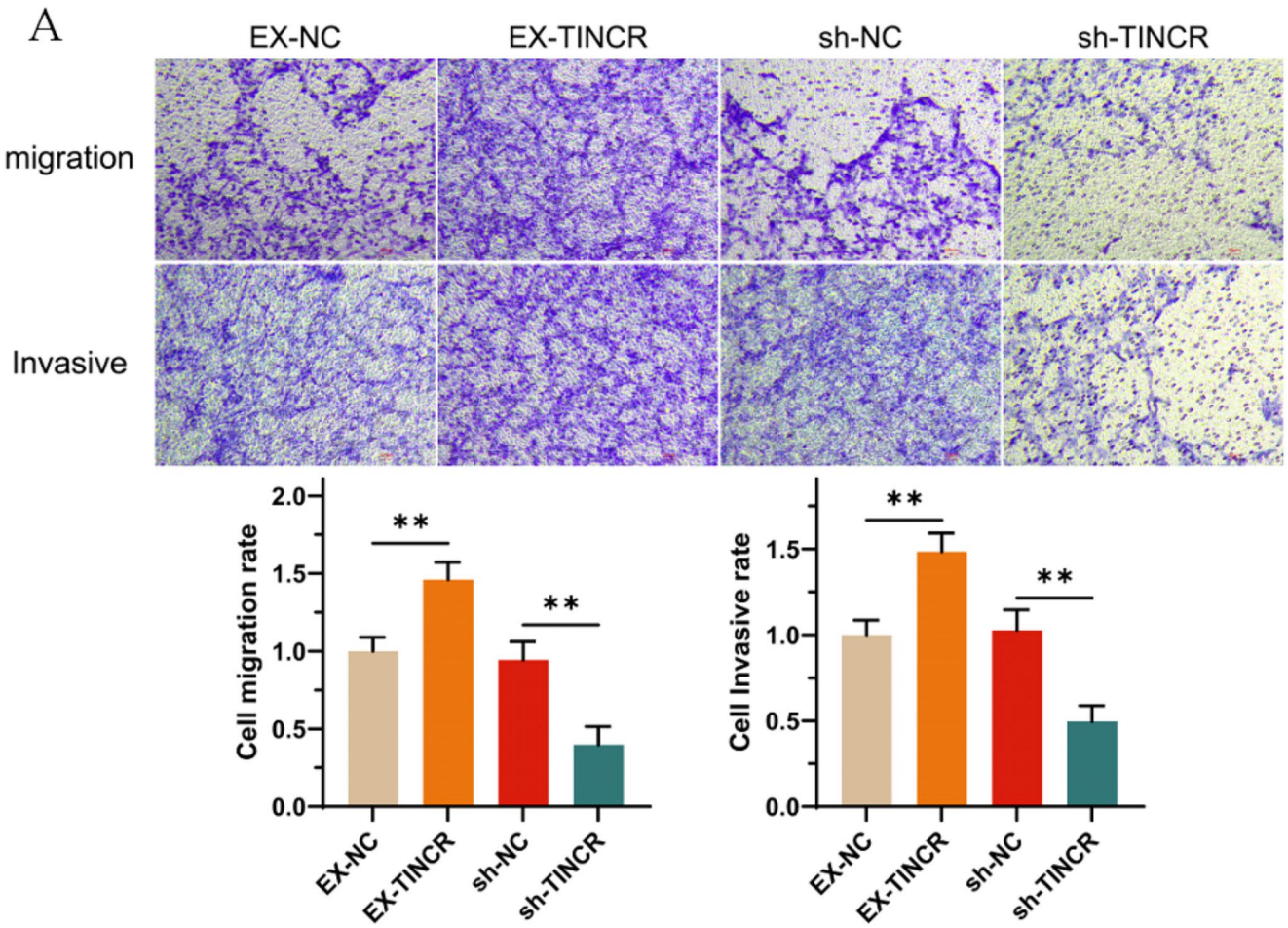

Fig. 2 Overexpression of TINCR significantly enhances the migration and invasion abilities of CSCC cells. **A**. Cell migration and invasion abilities are increased in the EX-TINCR group compared to the EX-NC group, while they are decreased in the sh-TINCR group compared to the sh-NC group. Experiments were repeated three times, and data are presented as mean ± standard error of the mean (SEM). *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$

metastasis and invasion processes of cutaneous squamous cell carcinoma.

TINCR inhibits Myc and TERC expression by increasing their methylation levels

RT-qPCR results showed that in A431 cells EX-TINCR group, the mRNA expression levels of N-MYC, L-MYC, c-MYC, and TERC genes were significantly higher than in the control group. Conversely, in A431 cells with TINCR knockdown, the expression levels of these genes were significantly lower than in the control group (Fig. 3A). These results indicate that lncRNA TINCR can significantly regulate the expression levels of the Myc gene family (including N-MYC, L-MYC, and c-MYC) and TERC in A431 CSCC cells. Both the Myc gene family and TERC are closely associated with cancer cell proliferation, migration, and invasion, suggesting that the role of lncRNA TINCR in regulating the behavior of CSCC cells may be partly achieved through the regulation of these genes. Upregulation of lncRNA TINCR may promote cancer cell growth, migration, and invasion by increasing the expression of Myc family genes and TERC. Conversely, knocking down TINCR may restrict these malignant behaviors by reducing the expression of these genes. These findings further validate the potential role of lncRNA TINCR as an oncogenic factor and suggest its potential therapeutic value in CSCC. Future studies could further explore the role of lncRNA TINCR in different types of cancer and evaluate its feasibility as a therapeutic target. The Western blot results (Fig. 3B) were consistent with the RT-qPCR findings, further demonstrating that lncRNA TINCR regulates the expression of these genes not only at the transcriptional level but also at the protein level.

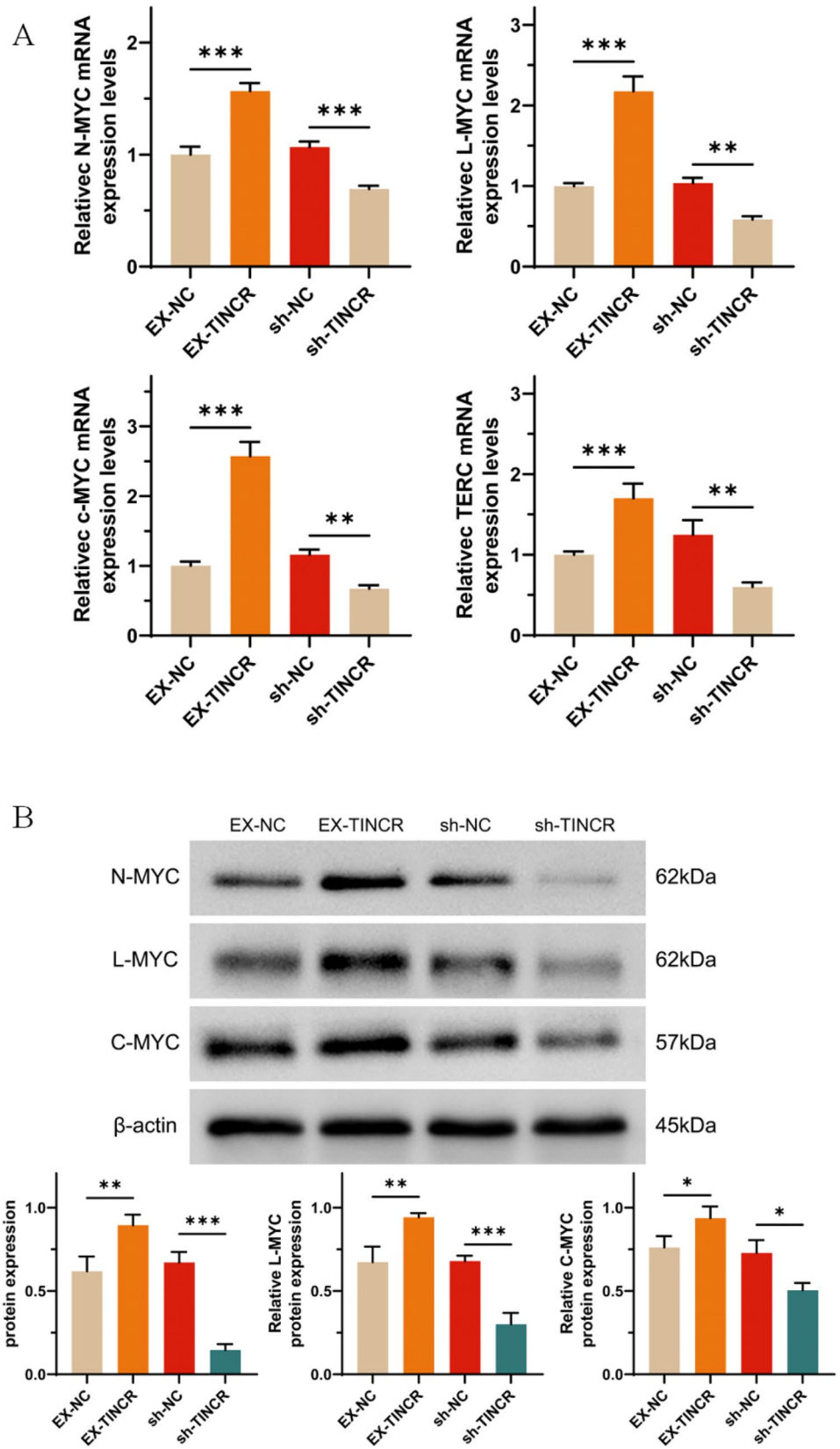

Fig. 3 TINCR inhibits Myc and TERC expression by increasing their methylation levels: (**A**) The mRNA expression levels of N-MYC, L-MYC, c-MYC, and TERC are significantly increased in the EX-TINCR group compared to the EX-NC group, while they are significantly decreased in the sh-TINCR group compared to the sh-NC group. (**B**) The protein expression levels of N-MYC, L-MYC, and c-MYC are significantly increased in the EX-TINCR group compared to the EX-NC group, while they are significantly decreased in the sh-TINCR group compared to the sh-NC group. Experiments were repeated three times, and data are presented as mean ± standard error of the mean (SEM). *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$

Overexpression of TINCR enhances the methylation levels of the Myc and TERC gene promoters, thereby inhibiting gene transcription

The Myc gene family and TERC are key components of oncogenes and telomerase, respectively, playing essential roles in tumor cell proliferation, migration, and invasion. Analyzing their methylation status can help evaluate whether lncRNA TINCR affects gene expression by regulating methylation, thereby modulating CSCC malignant behaviors. MSP results showed that in A431 cells EX-TINCR group, the methylation levels of the N-MYC, L-MYC, c-MYC, and TERC gene promoters were significantly higher than in the control group (EX-NC group), with methylation rates reaching 100%. Conversely, in A431 cells with TINCR knockdown, the promoter methylation levels were significantly reduced, with methylation rates ranging from 0 to 33.3% (Fig. 4A, B, C, D, E). These results indicate that lncRNA TINCR significantly regulates the methylation status of the Myc gene family (N-MYC, L-MYC, and c-MYC) and TERC gene promoters in A431 CSCC cells. Overexpression of TINCR significantly increased the methylation levels of these gene promoters, while knockdown of TINCR significantly decreased them. lncRNA TINCR may influence gene expression by altering the methylation status, thereby modulating the biological behavior of cancer cells.

TINCR promotes gene methylation by binding to DNMT1

The results of the RIP assay showed that in A431 cells EX-TINCR group, the binding of lncRNA TINCR to DNMT1 was significantly increased (compared to the EX-NC group, $P<0.001$). Conversely, in A431 cells with TINCR knockdown, the binding of lncRNA TINCR to DNMT1 was significantly reduced (compared to the sh-NC group, $P<0.001$). These results suggest that the expression level of lncRNA TINCR directly affects its binding affinity with DNMT1; lncRNA TINCR can directly bind to DNMT1, and this binding is significantly enhanced when lncRNA TINCR is overexpressed, while it is significantly weakened when TINCR is knocked down. DNMT1 is a key enzyme responsible for DNA methylation, mainly involved in the methylation of gene promoter regions, a modification typically associated with gene silencing [16]. The binding of lncRNA TINCR to DNMT1 may play a crucial role in regulating the promoter methylation status of the Myc gene family and TERC gene in cutaneous squamous cell carcinoma cells. These findings support the role of lncRNA TINCR as an epigenetic regulatory factor that, by binding to DNMT1, influences DNA methylation status and gene expression, thereby modulating cancer cell proliferation, migration, and invasion. Future studies could further investigate the specific mechanisms of TINCR-DNMT1 interactions, particularly their roles in different types of tumors, and assess the potential of targeting this interaction as a cancer therapeutic strategy (See Fig. 5).

Discussion

lncRNA TINCR plays a crucial role in the initiation, progression, and metastasis of tumors by regulating gene expression, RNA stability, protein interactions, and various other mechanisms. It participates in biological processes such as tumor cell proliferation, apoptosis, autophagy, migration, and invasion [6, 17]. For instance, in multiple solid tumors such as gastric cancer, breast cancer, and hepatocellular carcinoma, high expression of TINCR is associated with poor prognosis, and it functions through the competing endogenous RNA (ceRNA) mechanism by sponging microRNAs, thereby relieving their suppression on specific oncogenes [18–20]. Additionally, TINCR can mediate tumor cell sensitivity to chemotherapeutic agents by regulating epigenetic modifications and influencing the activity of signaling pathways [20]. Further investigation into the specific regulatory mechanisms of TINCR in various cancers and its potential clinical applications is of great importance for developing new diagnostic markers and therapeutic targets.

The pathophysiological mechanisms of CSCC are complex, involving factors such as gene mutations, epigenetic regulation, uncontrolled cell proliferation, and immune evasion [21]. UV-induced DNA damage leads to mutations in key tumor suppressor genes (such as TP53) and abnormal activation of oncogenes (such as c-MYC), driving tumor initiation and progression [2]. Moreover, epigenetic changes such as DNA methylation and histone modifications play critical roles in regulating CSCC-related gene expression and shaping the tumor microenvironment [14]. Although early detection of CSCC often leads to effective treatment, the high recurrence and metastasis rates necessitate the exploration of new molecular mechanisms and therapeutic targets to improve patient outcomes.

This study reveals the role of lncRNA TINCR in CSCC. The findings show that TINCR binds to DNMT1, promoting the methylation of the promoter regions of the Myc family genes (N-MYC, L-MYC, c-MYC) and the TERC gene, thereby inhibiting their expression. This mechanism effectively reduces the proliferation, migration, and invasion capacities of CSCC cells. The results of MSP and RIP experiments further support the role of TINCR in regulating gene methylation through its interaction with DNMT1. These findings suggest that lncRNA TINCR, as an epigenetic regulator, has tumor-suppressive potential in CSCC.

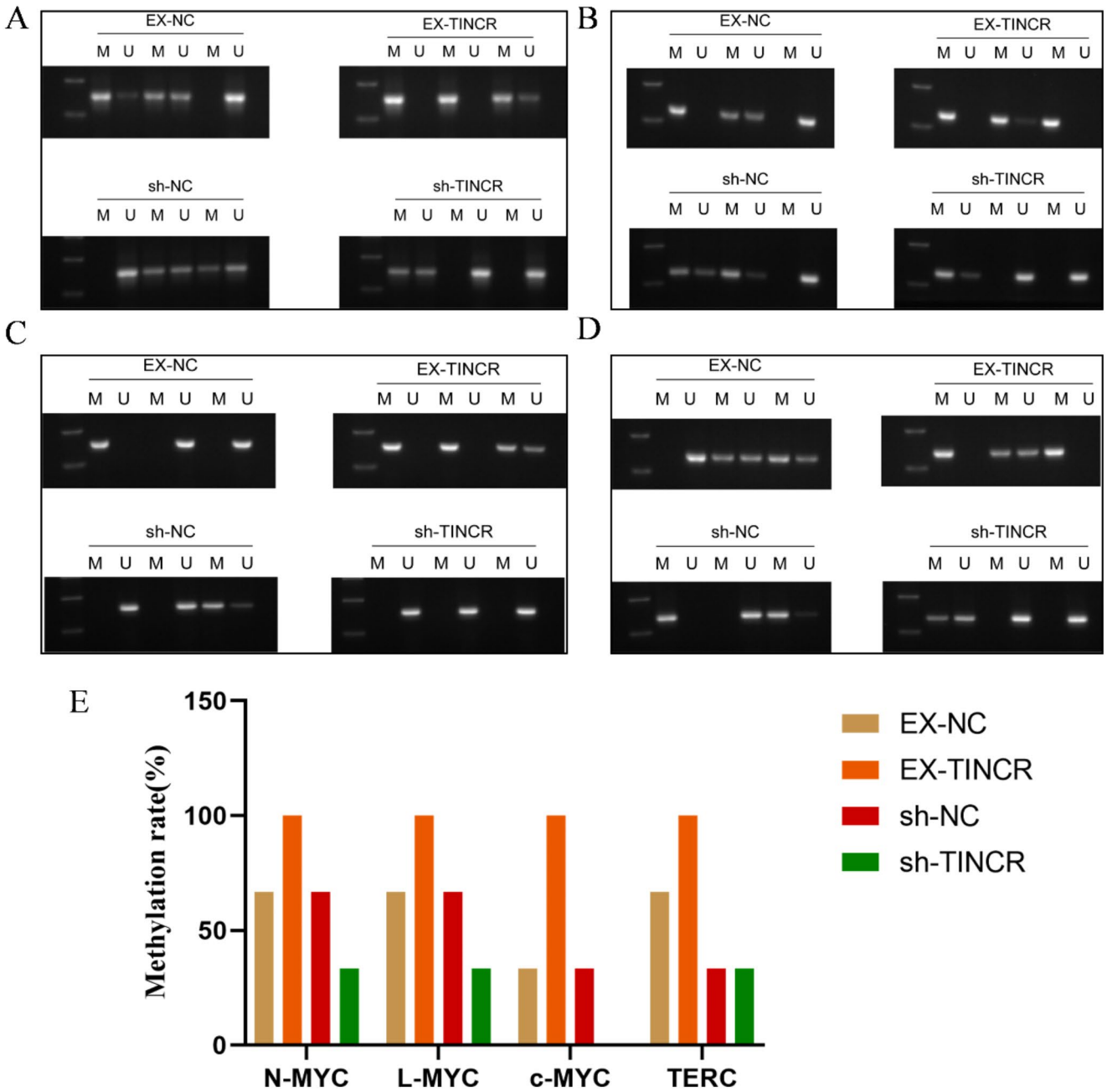

Fig. 4 MSP analysis of Myc (N-MYC, L-MYC, c-MYC) and TERC gene promoters: (**A**) MSP results for the N-MYC gene promoter: The methylation level (M) is significantly higher in the EX-TINCR group compared to the EX-NC group, and significantly lower in the sh-TINCR group compared to the sh-NC group. (**B**) MSP results for the L-MYC gene promoter: The methylation level is significantly higher in the EX-TINCR group compared to the EX-NC group, and significantly lower in the sh-TINCR group compared to the sh-NC group. (**C**) MSP results for the c-MYC gene promoter: The methylation level is significantly higher in the EX-TINCR group compared to the EX-NC group, and significantly lower in the sh-TINCR group compared to the sh-NC group. (D) MSP results for the TERC gene promoter: The methylation level is significantly higher in the EX-TINCR group compared to the EX-NC group, and significantly lower in the sh-TINCR group compared to the sh-NC group. (M: Methylated; U: Unmethylated). Experiments were repeated three times, and data are presented as mean ± standard error of the mean (SEM). *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$

The methylation levels of the Myc and TERC gene promoters play a crucial regulatory role in cancer development and progression [14]. Studies have shown that Myc regulates gene transcription inhibition by interacting with DNA methyltransferase DNMT3a [22]. Myc not only inhibits gene expression through its interaction with Miz-1 but also recruits DNMT3a to promote the methylation of tumor suppressor genes like p21, thereby suppressing their expression

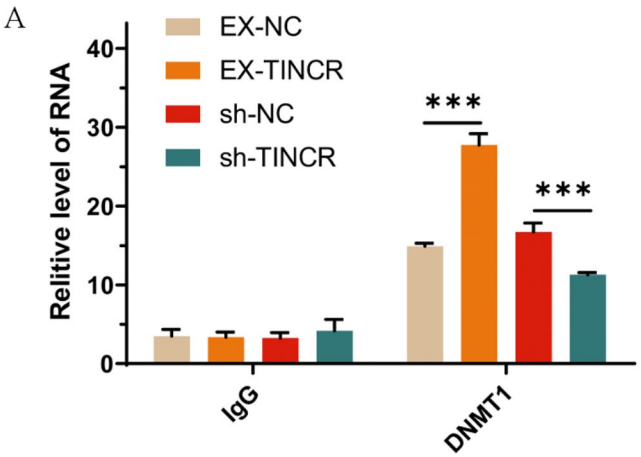

Fig. 5 RIP analysis of TINCR binding with DNMT1: The binding level of lncRNA TINCR to DNMT1 is significantly increased in the EX-TINCR group compared to the EX-NC group (***$P<0.001$) and significantly decreased in the sh-TINCR group compared to the sh-NC group (***$P<0.001$). No significant binding was observed with IgG control across all groups. Experiments were repeated three times, and data are presented as mean ± standard error of the mean (SEM). *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$

[23]. Myc is dependent on DNA methylation to repress downstream gene transcription, ultimately driving tumor development. The methylation regulation of the TERC gene is also closely related to cancer. As a critical component of telomerase, the methylation of TERC is essential for maintaining telomerase activity [13]. Research has shown that TERC methylation is regulated by the RNA-binding protein HuR, which promotes methylation at the C106 site of TERC, thereby increasing telomerase activity [24]. This mechanism is important for maintaining stem cell function and telomere length. Dysregulation of TERC methylation is associated with various cancers and human diseases.

TINCR inhibits the expression of the Myc gene in CSCC by promoting its methylation. Myc is a known oncogene that drives cancer progression by regulating the cell cycle and inhibiting apoptosis [25]. Studies on Myc have shown that it promotes methylation by recruiting DNMT1 or DNMT3a, leading to the suppression of tumor suppressor genes such as p21 [26]. While these studies highlight the importance of methylation regulation in cancer progression, TINCR's role is to inhibit Myc expression, in contrast to Myc's promotion of tumor cell growth. This represents a significant mechanistic distinction between the two. TERC and TINCR share the commonality of involving epigenetic regulation, particularly through DNA methylation to control downstream gene expression [27]. However, TERC's role within the telomerase complex is more associated with maintaining telomere length and anti-aging functions [28], whereas TINCR primarily suppresses oncogene expression via methylation, inhibiting tumor growth [6]. Both suggest the critical role of epigenetic modifications in cell proliferation, cancer, and aging, though their pathways and target genes differ.

This study reveals that TINCR exerts significant tumor-suppressive effects in CSCC by regulating the methylation of key oncogenes. This finding not only enhances our understanding of the molecular mechanisms underlying CSCC but also demonstrates that modulating epigenetic modifications can effectively control tumor development. Although in A431 cells overexpressing TINCR, we observed an increase in cell proliferation, migration, and invasion, a result that seemingly supports the role of TINCR as an oncogene, our study also suggests that TINCR may exert a complex dual effect by regulating the expression of Myc family genes and TERC gene through epigenetic mechanisms. Specifically, we found that overexpression of TINCR not only enhanced the methylation level of the promoter regions of Myc family genes (including N-MYC, L-MYC, and c-MYC) and TERC, but also that this increased methylation was closely associated with the suppression of these genes' expression. Myc family genes and TERC play critical oncogenic roles in cutaneous squamous cell carcinoma (CSCC), as they promote cell proliferation, inhibit apoptosis, and maintain the infinite proliferative capacity of tumor cells [29]. Overexpression of Myc genes is typically associated with tumor invasiveness and poor prognosis, while TERC, as a component of telomerase, maintains telomere length, supporting the continuous division and survival of tumor cells [30]. Therefore, controlling the expression of these genes has become a potential cancer therapy target. In our study, overexpression of TINCR enhanced the methylation levels of the Myc and TERC promoters and regulated the activity of these oncogenes by suppressing their transcriptional expression. Methylation, as an important epigenetic modification, is typically associated with gene silencing. Therefore, despite the fact that overexpression of TINCR promoted certain oncogenic features (such as proliferation and migration), we speculate that TINCR may promote methylation of the promoter regions of Myc family genes and TERC by interacting with DNA methyltransferases (such as DNMT1), thereby inhibiting their transcriptional activity and slowing down tumor proliferation and invasion. This suggests that TINCR may not only act as a pure oncogene but also potentially exert a tumor-suppressive effect through complex epigenetic regulation. By altering the methylation status of Myc family genes and TERC, TINCR could play a dual role in tumor cell proliferation, migration, and invasion, both promoting certain tumor features and limiting excessive growth by suppressing the expression of key oncogenes. This finding provides a new perspective on the complexity and diversity of epigenetic regulation in tumorigenesis and suggests potential future therapeutic strategies. Intervening

in the interaction between TINCR and methyltransferases, or directly regulating the methylation status of Myc family genes and TERC, could offer new treatment targets for CSCC and other tumor types.

Although this study reveals the mechanism by which lncRNA TINCR inhibits the proliferation, migration, and invasion of CSCC cells by promoting the methylation of Myc and TERC gene promoters, it does not fully elucidate the complex interactions between TINCR and other epigenetic regulators, such as DNA methyltransferases (DNMTs) [31], histone-modifying enzymes [32], and other long non-coding RNAs within the TINCR-mediated regulatory network. Future research should focus on several directions. First, further investigation is needed to explore how TINCR interacts with other epigenetic factors, particularly its regulatory relationships with DNA methylation enzymes (such as DNMT1) and demethylases, which will help provide a more comprehensive understanding of its role in gene expression regulation. Secondly, it is important to study whether TINCR is involved in the regulation of other cancer-related signaling pathways, such as the PI3K/AKT and Wnt/β-catenin pathways, to better understand its general and specific roles across different cancer types [33, 34]. Finally, based on these findings, therapeutic strategies targeting lncRNA TINCR, such as small molecule inhibitors, antisense oligonucleotides (ASOs), or RNA interference (RNAi) technologies, should be explored to inhibit its expression or function [35]. Additionally, the development of drugs specifically targeting TINCR, along with pharmacological and toxicological studies, is essential to determine its potential for clinical application. These research directions will not only enhance the understanding of the pathophysiological mechanisms of CSCC but may also provide new therapeutic avenues for treating CSCC and other related cancers.

Conclusion

This study reveals that lncRNA TINCR, by binding to DNMT1, promotes the methylation of the Myc and TERC gene promoters, thereby inhibiting the proliferation, migration, and invasion of CSCC cells. This mechanism provides new insights into the pathophysiology of CSCC and offers a new direction for the development of therapeutic strategies. Future research should further explore the role of TINCR in other cancers, particularly its extensive interactions with DNMT1 and its regulation of genomic methylation. Additionally, investigating the multifunctionality of TINCR in epigenetic mechanisms will help uncover its broader relevance in tumor biology and its therapeutic potential.

Statements and Declarations.

Acknowledgements The authors express their appreciation to staff in The First Affiliated Hospital of Harbin Medical University, for their technical assistance.

Author contributions Liang Wang: study design, data analysis, drafting the manuscript and revision of the manuscript. Yu Wang, Lei Xu: data collection and analysis, drafting the manuscript, investigation. All authors read and approved the final version of the manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors have no conflicts of interest to declare.

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