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ORIGINAL RESEARCH

RETRACTED ARTICLE: Long noncoding RNA UCA1 promotes the proliferation, invasion, and migration of nasopharyngeal carcinoma cells via modulation of miR-145

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Background: Nasopharyngeal carcinoma (NPC) is a common malignant tumor characterized by highly malignant local invasion and distant metastasis. Recently, increasing attention has been paid to long noncoding RNAs (lncRNAs), which play significant roles in tumorigenesis and progression. However, little is known about the potential role of the lncRNA urothelial carcinoma-associated 1 (UCA1) in NPC cell invasion and migration.

Methods: Real-time quantitative PCR was used to analyze the expression of lncRNA UCA1 in NPC cell lines and NP69. lncRNA UCA1 knock-down nasopharyngeal carcinoma cell line models were established through siRNA. Cell viability was evaluated by Cell counting kit-8 and Colony forming assay. The migration and invasion capacities were evaluated by wound healing and transwell migration and invasion assays. Western blot analysis were used to examine protein changes followed by UCA1 knock-down.

Results: Our study confirmed that UCA1 was upregulated in NPC cell lines and involved in NPC tumorigenesis according to our established UCA1-associated competing endogenous RNA network. Moreover, functional analyses indicated that the downregulation of UCA1 exerted inhibitory effects on cell proliferation, invasion, and migration. Mechanistic analyses revealed that UCA1 was the target of miR-145 and functioned as a sponge to repress miR-145 expression. Rescue experiments suggested that lncRNA UCA1 reversed the miR-145-mediated inhibition on oncogene ADAM17 expression, thus promoting the proliferation, invasion, and migration of NPC cells.

Conclusion: LncRNA UCA1 functions as a tumor promoter in NPC. UCA1 promotes the proliferation and invasion of NPC cells by sponging miR-145, functionally altering ADAM17 expression targeted by miR-145. Our exploration of the underlying mechanism of UCA1 in NPC may provide novel therapeutic targets for NPC.

Keywords: NPC, UCA1, miR-145, proliferation, invasion, migration

Introduction

Nasopharyngeal carcinoma (NPC), derived from the nasopharyngeal epithelium, is a common malignant tumor in Southeast Asia and Southern China.[1] With the advances in intensity-modulated radiation therapy and adjuvant chemotherapy, the long-term survival rate for NPC patients has been improved; however, local relapse and distant metastasis remain as the leading causes of mortality.[2] Therefore, the molecular mechanisms of NPC tumorigenesis and malignant progression need to be determined for effective diagnosis and therapy.

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Long noncoding RNAs (lncRNAs), which belong to a class of noncoding RNAs, comprise more than 200 nucleotides and are incapable of encoding proteins.[3] Emerging lines of evidence manifest that the deregulation of lncRNAs is involved in carcinogenesis and metastasis in many cancers and regulates several cancer-related processes, including cell proliferation, invasion, and migration.[4,5] Nevertheless, the mechanism of lncRNAs in tumor formation and development remains unclear. Several experimental studies have introduced the competing endogenous RNA (ceRNA) hypothesis, which states that lncRNAs can compete for common response elements of microRNAs (miRNAs) to serve as molecular sponges in regulating miRNA expression.[6] Liu et al[7] showed that the lncRNA Hox transcript antisense intergenic RNA drives the oncogenic growth of gastric cancer cells by downregulating miR-331-3p expression. Yuan et al[8] found that lncRNA-ATB functions as a sponge of the miR-200 family to suppress their functions, inducing the epithelial–mesenchymal transition (EMT), invasion, and metastasis of hepatocellular carcinoma. Collectively, we suppose that some lncRNAs may act as miRNA sponges that can affect cellular functions in NPC. The lncRNA urothelial carcinoma-associated 1 (UCA1), derived from chromosome 19p13.12, was found in a bladder tumor and contributes to oncogenic growth in many cancers, such as breast and gastric cancers.[9–11] However, the functions and underlying mechanisms of UCA1 in NPC development have not yet been investigated.

In this study, we evaluated whether UCA1 is upregulated in NPC cell lines and involved in NPC tumorigenesis. Moreover, we found that UCA1 functioned as a sponge of miR-145 to elevate the expression of oncogene *ADAM17*, thus promoting the proliferation, invasion, and migration of NPC cells.

Materials and methods

Cell culture

Five NPC cell lines (CNE-1, CNE-2, SUNE-1, 5-8 F, and 6-10B) and a human immortalized nasopharyngeal epithelial cell line (NP69) were purchased from the American Type Culture Collection. NP69 cells were maintained in keratinocyte/serum-free medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with bovine pituitary extract (BD Biosciences, Franklin Lakes, NJ, USA). These NPC cells were cultured in RPMI-1640 (Thermo Fisher Scientific) supplemented with 10% FBS (Thermo Fisher Scientific) in a humidified atmosphere of 5% CO_2 at 37°C.

RNA extraction and quantitative real-time PCR (qRT-PCR) assays

Total RNA was extracted from NPC cells by using TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's instructions to detect the expression levels of mRNAs. A reverse transcription reaction was conducted using an SYBR Green PCR Master Mix in the ABI7500 real-time PCR machine according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). The primer pairs used in this study are as follows: UCA1: 5′-CTCTCCATTGGC[illegible]ACCATTC-3′ and 5′-CTCTCCATTGGG[illegible]TCACC[illegible]TTC-3′; U6: 5′-CTCGCTTCGGCAGCA-C[illegible]-3′ and 5′-[illegible]ACGCTT CACGAATTTGCGT-3′; [illegible]DAM17: 5′-GC[illegible]TTCTCA AGTCTCCACAAG-[illegible] and [illegible]-CC[illegible]TTCGGGG CACATTCTG-3′; [illegible]actin: 5′-GGACTTCGAGC AAGAGATGG[illegible]3′ and 5′-AG[illegible]ACTGTGTTGG CGTACAG-[illegible]. The relative mRNA levels were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Cell transfection siRNAs targeting UCA1 (si-UCA1) and the negative control siRNA (si-NC) were obtained from RiboBio (Guangzhou, China). Moreover, a miR-145 inhibitor and miR negative control (inhibitor-NC) were synthesized by RiboBio. CNE-2 and 5-8 F cells were cultured in six-well plates (5×10^5 cells per well) at 24 hours prior to transfection. Then, the cells were transfected with si-UCA1/si-NC or miR-145 inhibitor/inhibitor-NC by Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's instructions. After 48 hours of transfection, the cells were harvested for further study.

Cell Counting Kit-8 (CCK8) and colony-formation assays

At 48 hours post-transfection, the cells were seeded in 96-well plates (3×10^3 cells/well) with five replicate wells for each group. A CCK8 assay (KeyGen, Nanjing, China) was used to determine cell viability at 1, 2, 3, or 4 days. The reaction solution (10 µL) was added to 100 µL of culture medium for each well and incubated at 37°C for 1 hour according to the manufacturer's instructions. The OD in each well was measured at a wavelength of 450 nm. The experiment was repeated thrice, and the results were treated as the average of these repeated measurements. For the colony-formation assay, the transfected cells were trypsinized, placed in a six-well plate (5×10^2), and cultured for 7–12 days. Then, the colonies were stained with 0.1% crystal violet with paraformaldehyde. The number of colonies was counted using ImageJ software. The experiments were conducted in triplicate.

Cell wound-healing and cell invasion and migration assays

The cell migration capacity was detected by a wound-healing assay. Transfected CNE-2 and 5-8 F cells were cultured in six-well plates and then starved of FBS for 24 hours. A wound was created by scratching the plate surface using a 200 µL pipette tube, and images of cell migration were taken at 0 and 24 hours by using an inverted microscope. For Transwell invasion and migration assays, the CNE-2 and 5-8 F cells were harvested and resuspended (2×10^4 cells/well) in serum-free medium and placed in the upper compartment of a chamber (Corning Inc., Corning, NY, USA) coated with or without a Matrigel membrane (BD Biosciences) 48 hours after transfection. Meanwhile, the media supplemented with 500 µL RPMI-1640 and 20% FBS were placed into the lower chamber. After incubation at 37°C for 12 or 24 hours, the migrated and invaded cells were fixed with paraformaldehyde and stained with crystal violet. Finally, five random fields of cells were counted randomly in each well.

Cell fractionation assay

We used the cell fractionation assay to detect the distribution of UCA1 in NPC cells by using the PARIS Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. U6 and β-actin were employed as the positive control for the nucleus and the cytoplasm, respectively.

Western blott analysis

Transfected NPC cells were washed twice with PBS before the addition of RIPA buffer (Beyotime, Shanghai, China). Protein concentrations were evaluated using a BCA protein assay kit. Protein (20 µg) was separated by 10% SDS-PAGE gels and shifted to a polyvinylidene difluoride membrane. Subsequently, the membranes were incubated with anti-ADAM17 (1:1,000; Cell Signaling Technology, Danvers, MA, USA), anti-E-cadherin (1:1,000; Cell Signaling Technology), anti-N-cadherin (1:1,000; Cell Signaling Technology), and anti-vimentin antibodies (1:1,000; Cell Signaling Technology). An anti-β-actin antibody (1:2,000; Cell Signaling Technology) was used as the loading control. Immunoreactive bonds were detected using the ECL detection reagent (Kaiji, Nanjing, China).

Luciferase reporter assay

The 293 T cells (3×10^5 cells per six-well plate) were co-transfected with either NC mimic or miR-145 mimic and the pCDNA3.1 luciferase reporter vector containing wild-type (WT) or mutant (mut) UCA1 using the Lipofectamine 2000 reagent (Thermo Fisher Scientific). They were harvested 48 hours after transfection, and their luciferase activities were evaluated using the Dual-Luciferase Reporter Assay System (Promega Madison, WS).

Statistical analysis

Statistical analysis was undertaken using the GraphPad Prism 5.0 software and SPSS 13.0. Comparisons between groups were carried out using Student's *t*-tests, and *P*-values <0.05 were considered statistically significant. All experiments were repeated for at least three times.

Results

LncRNA UCA1 was upregulated in NPC cells and connected with the tumorigenesis of NPC

To explore the correlation between lncRNA UCA1 expression and the tumorigenesis of NPC, we conducted qRT-PCR analysis to detect the expression of UCA1 in NPC cells. The results showed that the expression levels of UCA1 were upregulated in five NPC cell lines as compared with immortalized NP69 cells (Figure 1A). Among the NPC cell lines, CNE-2 and 5-8 F cells exhibited relatively high levels of UCA1; therefore, we chose these two NPC cell lines for further study. To predict the potential biological role of UCA1 in the carcinogenesis of NPC, bioinformatics analyses were employed on the basis of the emerging theme called ceRNA hypothesis. The hypothesis states that lncRNAs could function as molecular sponges to weaken miRNA expression by activating miRNA target genes.[6] In support of this point, we first tested the distribution of UCA1 in NPC cells and found that it was not only distributed in the cytoplasm but also in the nucleus (Figure 1B). Second, we utilized some database software (Miranda: http://www.microrna.org/microrna/home.do; Targetscan: http://www.targetscan.org/) to determine the paired miRNAs and mRNAs and construct the lncRNA UCA1-associated ceRNA network (Figure 1C). To understand the ceRNA network better, we used the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways to analyze the paired target genes. The GO pathways showed that the paired mRNAs were mainly enriched in functional clusters, including transcription, positive regulation of transcription from RNA polymerase II promoter, and signal transduction (Figure 1D). Meanwhile, the KEGG pathways exhibited that lncRNA UCA1-associated ceRNA targets were involved in axon guidance and cell adhesion molecules (Figure 1E). Some of

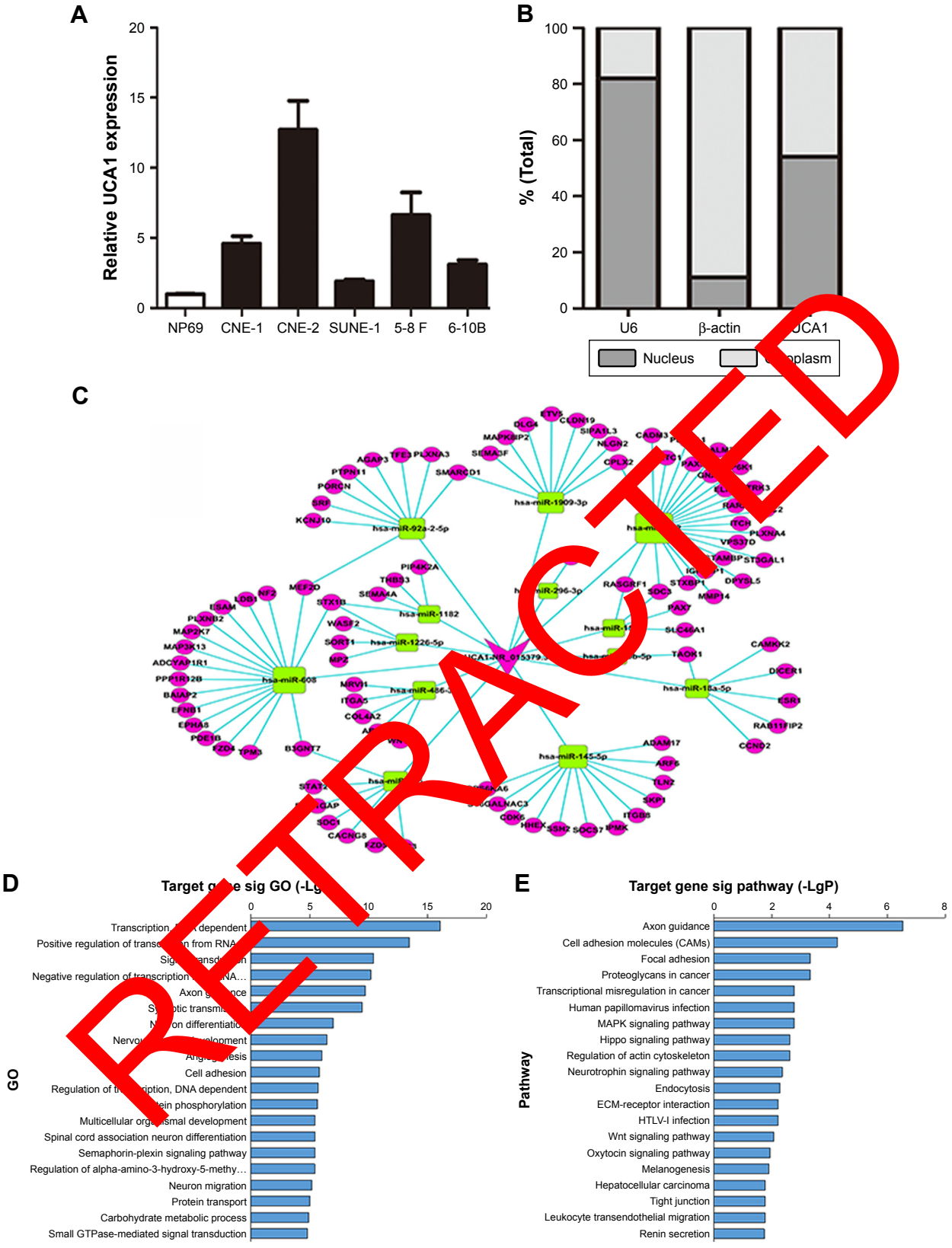

Figure 1 LncRNA UCA1 was upregulated in NPC cell lines and connected with the tumorigenesis of NPC.
Notes: (**A**) Expression levels of UCA1 in five NPC cell lines and NP69 cells were determined by qRT-PCR. (**B**) Subcellular locations of UCA1. U6 and β-actin were employed as positive controls in the nucleus and cytoplasm, respectively. (**C**) Established ceRNA network consists of UCA1, miRNAs, and their targets. (**D** and **E**) Significantly enriched GO (**D**) and KEGG (**E**) analyses of miRNA targets of the ceRNA network.
Abbreviations: NPC, nasopharyngeal carcinoma; qRT-PCR, quantitative real-time PCR; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; sig, significant.

the enriched signaling pathways, such as the focal adhesion, transcriptional misregulation in cancer, and MAPK signaling pathway, have been extensively reported to drive oncogenic growth in many cancers.[12–14] As discussed earlier, we found that lncRNA UCA1 was upregulated in NPC cells and possibly connected with tumorigenesis of NPC.

LncRNA UCA1 knockdown suppressed the proliferation, invasion, and migration of NPC cells

To further explore the possible biological functions of UCA1 in tumorigenesis, we transfected CNE-2 and 5-8 F cells with si-UCA1 or si-NC. Knockdown of UCA1 in CNE-2 and 5-8 F cells was determined through qRT-PCR (Figure 2A). Next, we employed CCK8 assays to determine the effects of UCA1 inhibition on the capability of cells to proliferate. The results suggested that UCA1 knockdown robustly caused inhibitory effects on cell viability (Figure 2B). Similar results were detected in colony-formation assays: UCA1 knockdown reduced cell proliferation (Figure 2C). Moreover, wound-healing assays showed that UCA1 knockdown delayed scratch healing in CNE-2 and 5-8 F cells compared with the controls (Figure 2D). In addition, Transwell assays with or without Matrigel demonstrated that

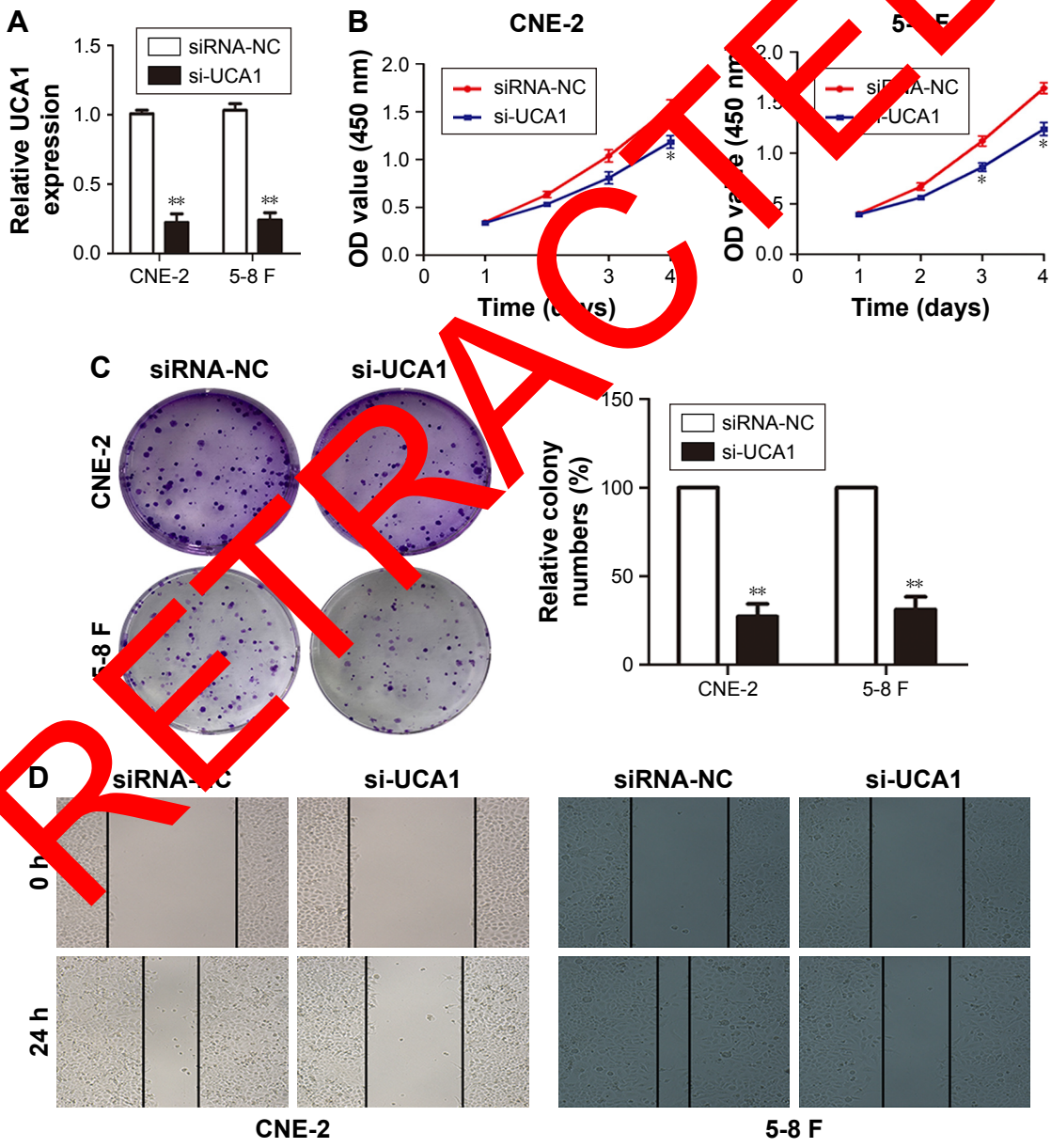

Figure 2 (*Continued*)

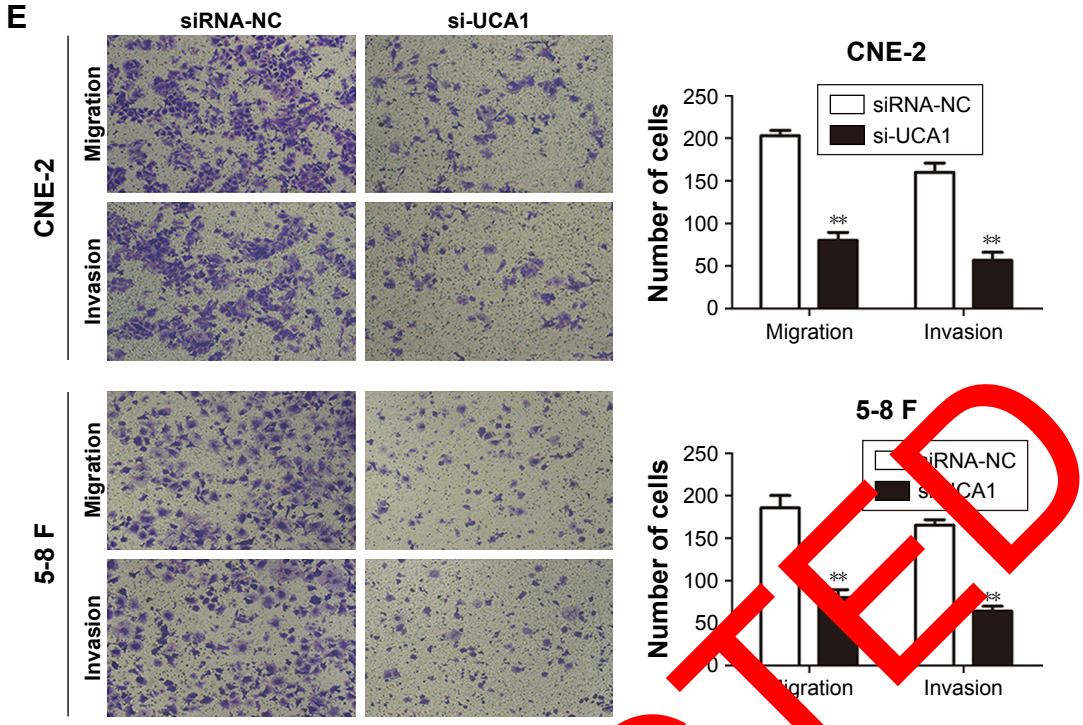

Figure 2 LncRNA UCA1 knockdown suppressed the proliferation, invasion, and migration of NPC cells.
Notes: (**A**) qRT-PCR was used to detect the efficiency of si-UCA1, $^{**}P<0.01$ vs control. (**B** and **C**) CCK8 and colony-forming assays were used to test cell proliferation, $^{*}P<0.05$, $^{**}P<0.01$ vs control. (**D**) Migration of NPC cells was determined by wound-healing analysis. (**E**) Transwell assays with or without Matrigel were employed to explore the invasion and migration of NPC cells, $^{**}P<0.01$ vs control. Original magnification ×200. Scale bars = 100 μm.
Abbreviations: NPC, nasopharyngeal carcinoma; qRT-PCR, quantitative real-time PCR; CCK8, Cell Counting Kit-8; NC, negative control.

UCA1 knockdown enhanced cell invasion and migration capabilities (Figure 2E).

LncRNA UCA1 acted as a molecular sponge for miR-145

LncRNAs regulate cancer progression partly by participating in the ceRNA regulatory network.[6] To determine whether lncRNA UCA1 has a similar mechanism in NPC, miR-145 was further studied according to our previous work.[15] Based on bioinformatics analysis, the 3′-UTR of lncRNA UCA1 included a putative binding site for miR-145 (Figure 3B). The expression levels of miR-145 were upregulated by knocking down the expression levels of lncRNA UCA1 in CNE-2 and 5-8 F cells (Figure 3A). These results showed that lncRNA UCA1 might play a role in the deregulation of miR-145. For further confirmation, we used dual-luciferase reporters containing UCA1, which contains WT or mut miR-145 binding sites, for target investigation. Our data showed that the co-transfection of miR-145 mimic significantly reduced the luciferase activities of the target region sequence of UCA1, whereas the luciferase activities of the mutant sequence of UCA1 showed no detectable change (Figure 3C).

LncRNA UCA1 modulated the proliferation, invasion, and migration of NPC cells by repressing the expression of miR-145

Our previous work provided evidence that miR-145 suppresses the proliferative capacity, invasion, and migration abilities of NPC cells.[15] Here, we investigated whether miR-145 reversed the promotion effects of UCA1 on cell proliferation, invasion, and migration. The CCK8 assay was conducted after co-transfecting CNE-2 or 5-8 F cells with si-UCA1 and miR-145 inhibitors. The data showed that the suppression of the proliferative capacity caused by knocking down UCA1 could be largely rescued by inhibiting miR-145 in NPC cells (Figure 4A). Meanwhile, the wound-healing assay demonstrated that the knockdown of UCA1 reversed the cell migratory capacities which were increased by the miR-145 inhibitor (Figure 4B). Additionally, Transwell analysis showed that UCA1 knockdown decreased the positive effect of the miR-145 inhibitor on cell invasion and migration in NPC cells (Figure 4C). Taken together, our data indicated that lncRNA UCA1 modulated the proliferation, invasion, and migration of NPC cells by repressing the expression of miR-145.

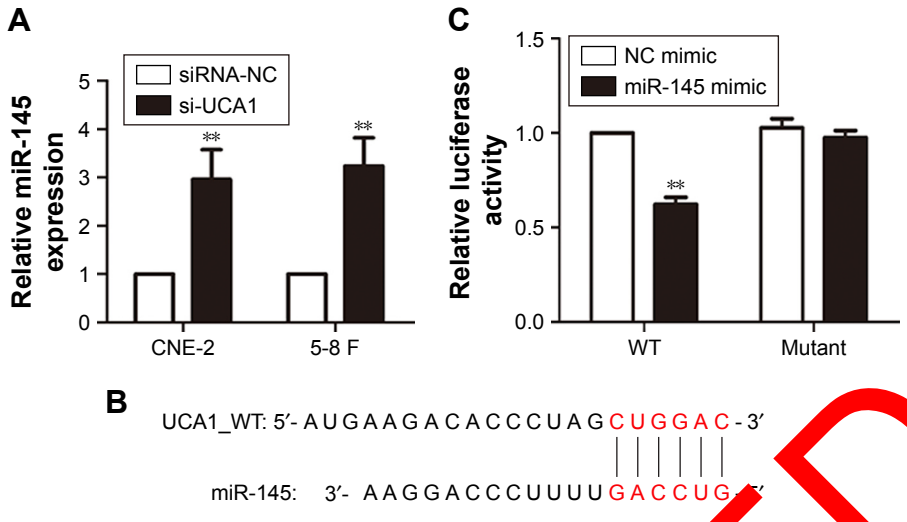

Figure 3 LncRNA UCA1 acted as a molecular sponge for miR-145.
Notes: (**A**) Expression of miR-145 in NPC cells transfected with si-UCA1 or siRNA-NC was detected by qRT-PCR, $^{**}P<0.01$ vs control. (**B**) The 3′-UTR of UCA1 contained the predicted binding site for miR-145. (**C**) Luciferase reporter assays exhibited that miR-145 significantly reduced the luciferase activity of the WT UCA1 instead of the mutant; $^{**}P<0.01$ vs control.
Abbreviations: NPC, nasopharyngeal carcinoma; qRT-PCR, quantitative real-time PCR; WT, wild type; NC, negative control.

LncRNA UCA1 increased ADAM17 expression by inhibiting miR-145

Previous research demonstrated that miR-145 represses tumorigenesis in NPC by directly targeting ADAM17 – a migration-related protein in many cancers.[15] In this study, we confirmed that the mRNA and protein levels of ADAM17 were reduced by si-UCA1 treatment (Figure 5A). Xu et al[16] reported that ADAM17 overexpression could promote EMT

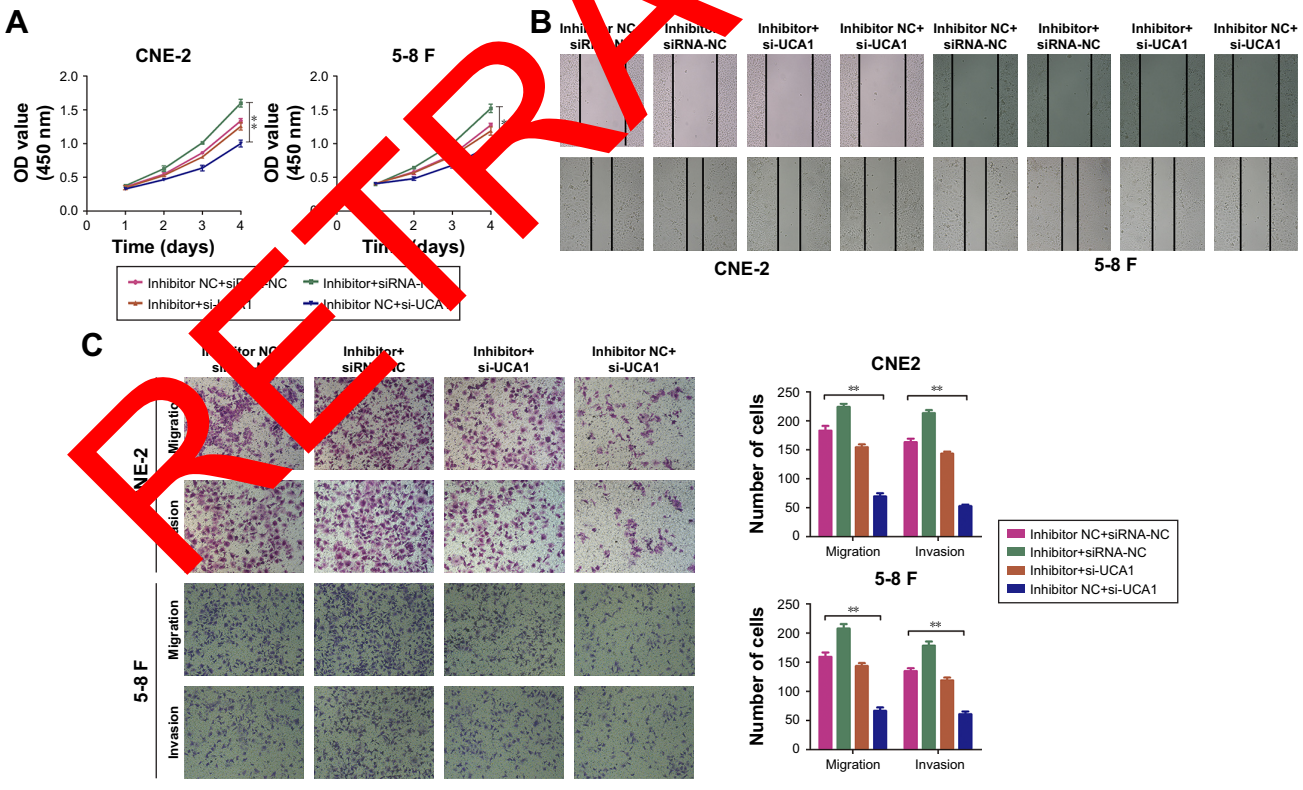

Figure 4 LncRNA UCA1 modulated the proliferation, invasion, and migration of NPC cells by repressing the expression of miR-145.
Notes: (**A**) CCK8 analysis was used to examine the effect of UCA1 and miR-145 on cell viability, $^{**}P<0.01$ vs control. Wound-healing (**B**) and Transwell analyses (**C**) were used to exhibit the effect of UCA1 and miR-145 on cell invasion and migration, $^{**}P<0.01$ vs control. Original magnification ×200. Scale bar 100 μm.
Abbreviations: NPC, nasopharyngeal carcinoma; CCK8, Cell Counting Kit-8; NC, negative control.

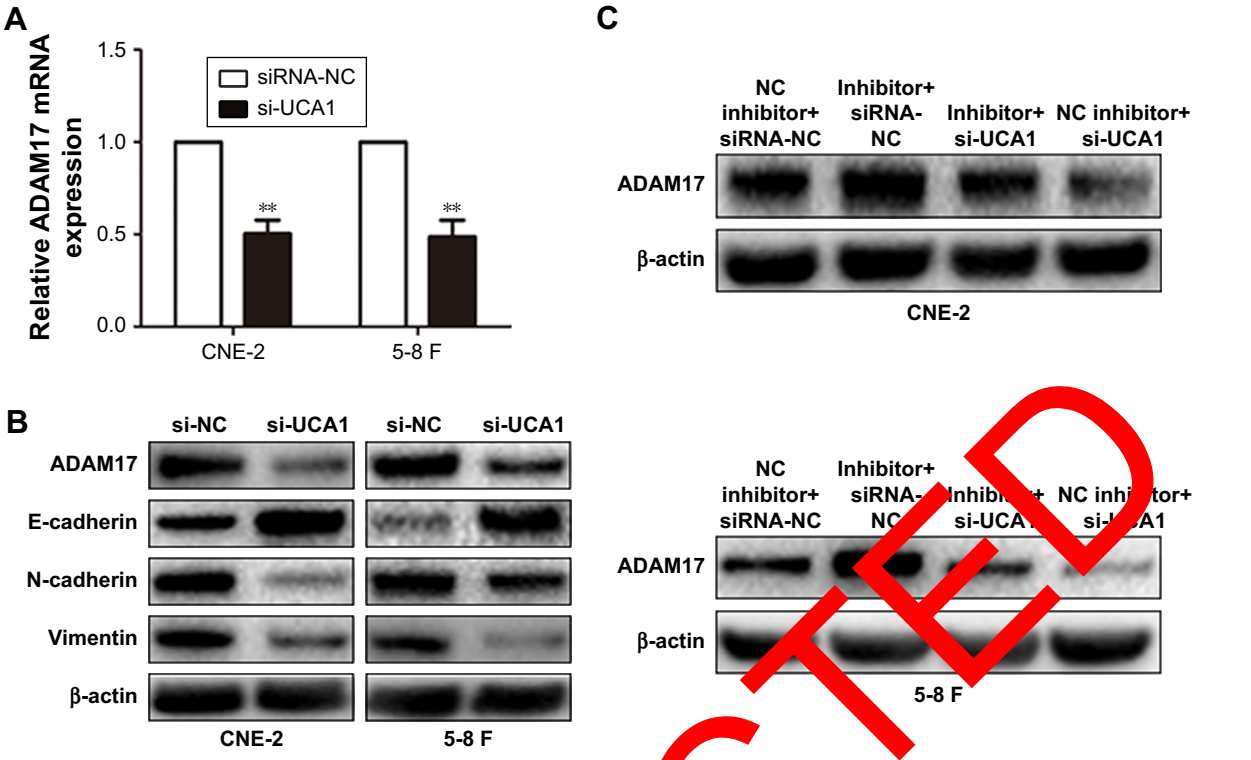

Figure 5 LncRNA UCA1 increased ADAM17 expression by inhibiting miR-145.
Notes: (**A**) ADAM17 expression was detected by qRT-PCR, **$P<0.01$ vs control. (**B**) Western blott analysis showed that UCA1 knockdown decreased the protein levels of ADAM17 and EMT-related markers. (**C**) Western blott analysis showed the expression of ADAM17 in NPC cells transfected with si-UCA1 and miR-145 inhibitor.
Abbreviations: qRT-PCR, quantitative real-time PCR; EMT, epithelial–mesenchymal transition; NPC, nasopharyngeal carcinoma; NC, negative control.

in gastric cancer cells by activating the TGF-β/SMAD signaling pathway. Therefore, we detected the expression of epithelial markers such as E-cadherin and mesenchymal markers such as N-cadherin and vimentin through Western blott analysis. The results showed that UCA1 knockdown significantly increased the expression of E-cadherin and reduced the expression of N-cadherin and vimentin at the protein level in NPC cells (Figure 5B).

Subsequently, we explored whether lncRNA UCA1 could increase ADAM17 expression by inhibiting the expression of miR-145. We co-transfected CNE-2 or 5-8 F cells with si-UCA1 and miR-145 inhibitors. The results showed that the downregulated expression of UCA1 inversed the protein levels of ADAM17, which were promoted by the inhibition of miR-145 (Figure 5C). Ultimately, our data provided the foundational basis that lncRNA UCA1 could increase ADAM17 expression by serving as a molecular sponge for miR-145.

Discussion

Recently, studies on lncRNAs, which play important roles in multiple cancer pathogenesis, have increased.[17,18] UCA1, a highly conserved lncRNA, is notably overexpressed in diverse types of cancers and promotes gastric, pancreatic, glioma, and endometrial carcinoma cell proliferation and metastasis.[19–22] In this study, we found that UCA1 expression was also upregulated in NPC cells, and high UCA1 levels were correlated with the tumorigenesis of NPC. Moreover, the biological function of UCA1 in NPC cells was investigated. Our data indicated that UCA1 promoted NPC cell proliferation, invasion, and migration, thus playing an oncogenic role in nasopharyngeal cancer. However, the mechanistic basis for the involvement of UCA1 in NPC development remains unclear.

Several attempts have been made to detect the increasing physiological functions of lncRNAs. Specific endogenous lncRNAs that contain miRNA binding sites can function as ceRNAs to sponge up specific miRNAs and interfere with their functions, thereby regulating gene expression.[23,24] For example, NEAT1_2 – an lncRNA that is overexpressed in papillary thyroid cancer – can regulate ATAD2 expression by competitively binding to miR-106b-5p.[25] Similarly, LINC01234 promotes the depression of core-binding factor-β by acting as a ceRNA for miR-204-5p, thereby leading to the development of gastric cancer.[26] Additionally, lncRNA-UCA1 regulates cell proliferation and invasion in pancreatic cancer by serving as a molecular sponge for miR-135a.[20] Here, we hypothesized that UCA1 may enhance NPC cell proliferation, invasion, and migration by targeting specific miRNAs.

According to our previous work[15] and the established ceRNA network, we chose miR-145 for further study. The expression levels of miR-145 were detected by qRT-PCR after silencing lncRNA UCA1 in CNE-2 and 5-8 F cells, and the results showed an obvious upregulation of miR-145. Dual-luciferase reporter assays were conducted because a putative binding site for miR-145 was included in the 3′-UTR of UCA1. The results confirmed that UCA1 was the target of miR-145. Moreover, rescue experiments showed that the miR-145 inhibitors reversed the tumor-promoting function of UCA1 knockdown on NPC cells.

Existing studies recognized the critical role of miR-145 in regulating cell death in cancers.[27] MiR-145 inhibits the invasion of gastric cancer cells by downregulating CTNND1 expression and altering the location of CTNND1 and E-cadherin.[28] Moreover, miR-145 has been reported as a tumor-suppressive RNA that directly targets adducin 3 and Sox9 in human glioma cells.[29] Our previous work provided evidence that miR-145 represses the proliferation, invasion, and migration of NPC cells by targeting ADAM17 in the modulation of EGFR and E-cadherin. Furthermore, the overexpression of ADAM17 can activate the TGF-β/SMAD signaling pathway, which induces EMT in gastric cancer cells.[16] By using qRT-PCR and Western blott analysis, the [illegible] and protein levels of ADAM17 decreased with the treatment of si-UCA1. Furthermore, the results exhibited the upregulated expression of E-cadherin and downregulated expression of N-cadherin and vimentin at the protein level with UCA1 knockdown. To demonstrate whether lncRNA UCA1 could increase the expression of ADAM17 by inhibiting the effect of miR-145, co-transfection with si-UCA1 and miR-145 inhibitor was conducted. The data showed that the downregulated expression of UCA1 could reverse the functions of miR-145 in CNE-2 and 5-8 F cells. Taken together, lncRNA UCA1 could increase ADAM17 expression and activate EMT by serving as a molecular sponge for miR-145.

Conclusion

We have identified that lncRNA UCA1 functions as a tumor promoter in NPC. UCA1 promotes the proliferation and invasion of NPC cells by sponging miR-145, functionally altering ADAM17 expression targeted by miR-145, and inducing the EMT process. Our exploration of the underlying mechanism of UCA1 in NPC may provide novel therapeutic targets for NPC.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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