Role of lncRNA-ENST00000412010 in regulating nasopharyngeal cancer cell survival*

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is a rare tumor with highly recurrent and lack of effective treatment. Long non-coding RNAs (lncRNAs) have been reported to play roles in various cancers including NPC.

Methods: In the current study, two cell lines of NPC (CNE-2Z and 5-8F cells) were transfected with short hairpin RNA (shRNA) targeting lncRNA-ENST00000412010 (shlncRNA) or control shRNA (shControl). Cell proliferation, survival, in vitro colony formation, and in vivo xenograft tumor formation were then investigated.

Results: The study found that cells transfected with shlncRNA grew significantly slower than the cells transfected with shControl as measured on day 5; increased in Annexin V expression; decreased in colony formation; and smaller in xenograft tumor size on day 45. Expression of DNA damage-inducible transcript 3, dual specificity protein phosphatase 5, insulin receptor substrate 1, interleukin-6, and tribbles homolog 3 genes was significantly up-regulated in the cells transfected with shlncRNA, while gene expression of matrix metalloproteinase-7 and cyclin-dependent kinase 4 inhibitor B was significantly down-regulated in the cells transfected with shlncRNA. Immunoblotting assay confirmed DUSP5 protein was significantly increased while proteins of MMP-7 and CDKN2B were significantly lower in the cells lacking lncRNA than that of the control cells.

Conclusions: These findings suggested that lncRNA-ENST00000412010 plays a role in modulating NPC survival and tumorigenesis through regulating molecules associated with cell cycle and protein phosphatase.

Key words: nasopharyngeal carcinoma, long non-coding RNAs (lncRNA), modulating tumorigenesis

Introduction

Nasopharyngeal carcinoma (NPC) is a rare, but highly malignant tumor. While NPC incidence has declined gradually in the last decades [1], lack of effective treatment for NPC results in high rates of tumor recurrence and distant metastasis [2]. Linkage and association studies have identified long non-coding RNAs (lncRNAs) may contribute to the tumorigenesis of NPC.

Long non-coding RNAs (lncRNAs) are generally defined as transcripts longer than 200 nucleotides, but are not protein-coding [3]. Recent studies indicated that over 15,000 lncRNAs encoded by the human genome, suggesting lncRNAs are ubiquitous in mammalian genomes [4]. While lncRNAs could not be translated into proteins, the majority of them play a key role in many biological processes as well as pathogenesis of variety kinds of diseases including tumorigenesis and progression of cancers [5-8]. In this regard, several lncRNAs including EWSAT1 [9], LINC00460 [10], HOTAIR [11], C22orf32-1 [12], PXN-AS1-L [13], PVT1 [14], LINC01133 [15], DANCR [16], and FAM225A [17] haven been reported to be associated with NPC tumorigenesis.

To screen potentially implicated lncRNA in the NPC, we searched the GEO database on "Expression of mRNAs and lncRNAs in cancer revealed by microarray" (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70880) and selected LncRNA-ENST00000412010 as potential target. LncRNA-

ENST00000412010 is located at chromosome 9, and pseudogene similar to part of ribosomal protein L7 (RPL7). Preliminary experiments demonstrated that LncRNA-ENST00000412010 is expressed in the NPC. In the current study, therefore, we hypothesized that lncRNA-ENST00000412010 may play a role in the tumorigenesis of nasopharyngeal carcinoma through regulating cell survival and proliferation. To accomplish this, a cell line of nasopharyngeal carcinoma (CNE-2Z) was transfected with shRNA targeting lncRNA-ENST00000412010 or control-shRNA followed by investigating cell survival of the NPC cell line and in vivo xenograft formation.

Materials and methods

Ethics approval and consent to participate

This research was approved by the Ethics Committee of Changzheng Hospital, The Second Military Medical University. All methods were carried out in accordance with relevant guidelines and regulations. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Materials

The CNE-2Z cell line of nasopharyngeal cancer was purchased from BeNa Culture collection (Beijing, China). The 5-8F cell line of nasopharyngeal cancer was purchased from Shanghai Genechem Co.,Ltd (Shanghai, China). GV493 vector, pHelper1.0 vector, and pHelper2.0 vector were purchased from Shanghai Genechem Co.,Ltd (Shanghai, China).

Cell line and cell culture

Nasopharyngeal cancer cell lines, CNE-2Z was cultured with DMEM medium supplemented with 10% fetal calf serum (FCS), and the 5-8F cell line was cultured with RPMI-1640 supplemented with 10% FCS.

Construction and transfection of shControl and shlncRNA

GV493 vector (as shown Supplement Figure 1) was used to construct the vector that contained the sequences as shown below (Table 1), which could specifically target lncRNA-ENST00000412010 gene.

Lentivirus vectors containing negative control RNAi sequence (TTCTCCGAACGTGTCACGT, shControl) [18-20] or the RNAi sequence targeting lncRNA-ENST00000412010 (shlncRNA) were constructed by co-transfecting 293 T cells with GV493 vector containing either scramble sequence or psc66106-1 sequence, pHelper1.0 vector (Supplement Figure 2A), and pHelper2.0 vector (Supplement Figure 2B). They were named as pFU-GW-016PSC53349-1 (shControl) and LVpFU-GW-016PSC66106-1 (shlncRNA), respectively.

Cells (2 x 10^5/mL) were plated into 6-well plate (2mL/well) and cultured till 80% confluence in 10% FCS-DMEM medium. Cells were then transfected with either control lentivirus, shControl (pFU-GW-016PSC53349-1) or shlcRNA-ENST00000412010 (LVpFU-GW-016PSC66106-1) at titration of 5x10^8 TU/mL) for 8 hours. After transfection, efficiency of the transfection was determined either by fluorescence microscopy (Supplement Figure 3) or real time RT-PCR (Supplement Figure 4) without puromycin selection.

Cell proliferation assay

After transfection with either shControl or shlncRNA, the cells were plated into 96-well plate, at a density of 2500 cells/well/100µL medium (10%FCS-DMEM) in triplicated wells. Cell number was counted daily for 5 days using Celigo Cytometer (Nexcelom Bioscience, Lawrence, MA, USA) following the manufacture's instruction.

Apoptosis assay

Three days after transfection with either shControl or shlncRNA, the cells were plated into 6-well plates and cultured for additional 2 days to allow the cells became 85% confluent. Cells were then harvested by trypsinization and stained for Annexin V-APC following the manufacture's instruction (eBioscience, cat #: 88-8007, San Diego, CA, USA). Apoptosis was then analyzed by flow cytometry.

MTT assay

Three days after transfection with either shControl or shlncRNA, the cells were plated into a 96-well plate at a density of 2000 cells/well. On the day of assay, 20µL MTT (5mg/mL) was added into each well and incubated for 4 h. After brief rinse with PBS once, 100µL DMSO was added into each well. After 5 min shaking, the plate was read at 490/570 nm wave-length. OD value was compared.

Colony formation and clonogenic assays

Three days after transfection with either shControl or shlncRNA, the cells were plated into 6-well plates at a density of 400 cells/well. Cells were cultured for 12 days with medium change every 3 days. After washing once with PBS, cells were fixed and stained

Table 1. RNAi sequences that targeting lncRNA (ENST00000412010 gene).

Name	5′-addition	STEM	Loop	STEM	3′-addition
psc66106-1	CCGG	GACCAGATCAACAGCCTTCTT	CTCGAG	AAGAAGGCTGTTGATCTGGTC	TTTTTG
psc66106-2	AATTCAAAAA	GACCAGATCAACAGCCTTCTT	CTCGAG	AAGAAGGCTGTTGATCTGGTC	

with Kwik Diff kit (ThermoFisher Scientific, Shanghai, China). Colonies were counted under microscope at 40 x magnification.

Subcutaneous nasopharyngeal tumor xenograft formation study

All animal studies were performed following the Institutional Regulation Guidelines, and were approved by the Shanghai Science and Technology Commission (IRB#: SCXK 2018-0003). BALB/c nude mice (4 weeks old) were purchased from Shanghai Lingchang Biotechnology (Shanghai, China). Before the animals were injected with tumor cells, the cells were transfected with shControl or shlncRNA as described above. Cells with 80% or better transfection were used for injection into the animals. Subcutaneous xenografts were established by subcutaneous injection of shControl cells or shlncRNA-ENST00000412010 (CNE-2Z) cells (200μL of 2 x 10^7 cells/L, that is, 4 x 10^6 cells total) into the right flank of nude mice and allowed to grow for 45 days. Tumor dimensions were determined using caliper measurement on day 29, 34, 38, 42, and 45. After 45 days, mice were killed, and the tumors were extracted and weighted.

Immunoblotting

Three days after transfection with either shControl or shlncRNA, the CNE-2Z cells were plated into 60 mm dishes. After 2 days of culture, cells were washed once with cold PBS. Cell lysis buffer (1M Tris-HCL, pH 6.8, 2% 2% ß-mercaptoethanol, 20% glycerin, 4% SDS) was added (500μL/dish) and cells were scrapped off. After brief sonication, the cell lysates were centrifuged at 12000 g x 15min at 4°C. The supernatants were collected and protein concentration was determined by BCA methods. Proteins (10μg/lane) were separated by 10% SDS-PAGE gels and transferred to PVDF membrane. After blocking with 5% milk-PBS for 1 h at room temperature, the following primary antibodies were applied following the manufacture's instruction and incubated at 4°C overnight: IRS1 (Abcam, Cat#: ab52167, Shanghai, China), IL-6 (Abcam, Cat#: ab6672, Shanghai, China), DDIT3 (Abcam, Cat#: ab11419, Shanghai, China), TRIB3 (Abcam, Cat#: ab75846, Shanghai, China), CDKN2B (Abcam, Cat#: ab53034, Shanghai, China), MMP-7 (Abcam, Cat#: ab4044, Shanghai, China), DUSP5 (Abcam, Cat#: ab54939, Shanghai, China), and GAPDH (Santa Cruz, sc-32233, Dallas, TX, USA). Next day, after washing, appropriate 2nd antibodies were added and incubated for 1h at room temperature. After washing, the membrane was subjected to hypersensitive reagents before the exposure to X-ray and the development. GAPDH was used as internal reference. The protein bands were scanned and analyzed with a molecular biological image analysis software to determine the protein levels.

Real time RT-PCR

Three days after transfection with either shControl or shlncRNA, the CNE-2Z cells were plated into 60 mm dishes. After 2 days of culture, cells were harvested using Trizol. Total RNA was extracted and cDNA was synthesized using reverse transcription kit. Real-time quantitative RT-PCR was performed using primers & probe sets as well as reagents from commercial source (Applied Biosystems, Foster City, CA, USA). GAPDH was used as an endogenous control. The sequences of the primers used for the PCR were listed in Table 2. Expression of each gene of interested (GOI) was normalized to the housekeeping gene (HKG) GAPDH. Relative mRNA levels were calculated as $2^{-\Delta Ct} = 2^{-(Ct\ (HKG) - Ct\ (GOI))}$.

Statistical analysis

All data were shown as the mean ± SD of triplicate values from at least three independent experiments. The significance of the difference between groups was determined with t-test using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered as significant.

Results

Suppression of lncRNA-ENST00000412010 gene resulted in inhibition of nasopharyngeal cancer cell growth

As shown in Figure 1, the CNE-2Z cells transfected with shlncRNA grew significantly slower than the cells transfected with shControl (Figure 1A), the difference was statistically significant on day 3 (5.85 ± 0.38 of shControl vs 2.02 ± 0.20 of shlncRNA, $P < 0.05$, Figure 1C), day 4 (8.64 ± 0.25 of shControl vs 2.62 ± 0.11 of shlncRNA, $P < 0.05$, Figure 1C), and day 5 (11.04 ± 0.38 of shControl vs 2.93 ± 0.12 of shlncRNA, $P < 0.05$, Figure 1C). Similarly, the 5-8F cells transfected with shlncRNA grew significantly slower than the cells transfected with shControl (Figure 1B), and the difference was time-dependently increased (Figure 1D).

Suppression of lncRNA-ENST00000412010 gene led to apoptosis

As shown in Figure 2, suppression of lncRNA-ENST00000412010

Table 2. Sequences of the primers.

Genes	Primer sequence	Size (bp)
GAPDH	Forward: TGACTTCAACAGCGACACCCA Backward: CACCCTGTTGCTGTAGCCAAA	121
IL-6	Forward: CAAATTCGGTACATCCTCG Backward: CTCTGGCTTGTTCCTCACTA	259
CDKN2B	Forward: CTGGACCTGGTGGCTACG Backward: ACATTGGAGTGAACGCATCG	114
DUSP5	Forward: TCCTCACCTCGCTACTCG Backward: ACATCCACGCAACACTCAG	106
MMP-7	Forward: GGGGACTCCTACCCATTTG Backward: TCCAGCGTTCATCCTCATC	106
IRS1	Forward: ACAAACGCTTCTTCGTACTGC	158
DDIT3	Forward: CACTCTTGACCCTGCTTCTCTG Backward: TTTCCGTTTCCTGGTTCTCC	160
TRIB3	Forward: AAGCGGTTGGAGTTGGATGAC Backward: CACGATCTGGAGCAGTAGGTG	127

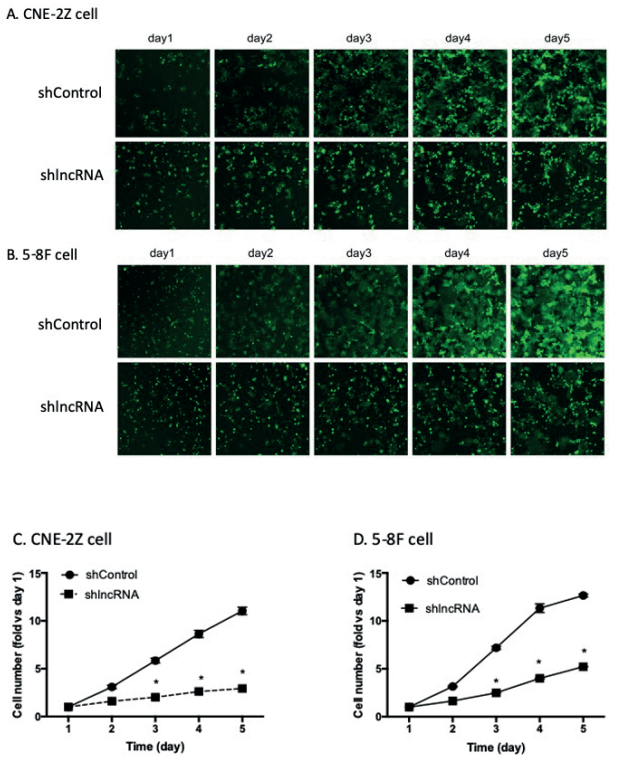

Figure 1. Effect of lncRNA-ENST00000412010 on cell proliferation. A) Representative images of 5-day proliferation of CNE-2Z cell line. B) Representative images of 5-day proliferation of 5-8F cell line. C) and D) Quantitative comparison of cell number in the CNE-2Z (C) and 5-8F (D) cells transfected with shControl or shlncRNA. Vertical axes: cell number expressed as fold change compared to day 1; horizontal axes: time (day). * P < 0.05 compared to shControl cells at corresponding time point.

resulted in significant increase of apoptosis in the CNE-2Z cells (6.68 ± 0.58 vs 3.31 ± 0.23 of shControl cells, P < 0.01, Figure 2A and B) as well as in the 5-8F cells (8.56 ± 0.46 vs 4.50 ± 0.21 of shControl cells, P < 0.01, Figure 2C). Suppression of cell proliferation following lncRNA transfection was further confirmed by MTT assay (Figure 3), which demonstrated that CNE-2Z cells transfected with lncRNA-ENST00000412010 grew significantly slower than that of the cells transfected with shControl on day 3 (0.28 ± 0.01 vs 0.56 ± 0.01, P < 0.05), day 4 (0.42 ± 0.01 vs 1.01 ± 0.01, P < 0.05), and day 5 (0.618 ± 0.01 vs 1.28 ± 0.01, P < 0.05, Figure 3A). Similar result was found in the 5-8F cells (Figure 3B).

Suppression of lncRNA-ENST00000412010 gene resulted in inhibition of colony formation and xenograft tumor growth

As shown in Figure 4, in vitro cell culture demonstrated that suppression of lncRNA-ENST00000412010 gene resulted in inhibition of colony formation in the CNE-2Z cells (Figure 4A) and 5-8F cells (Figure 4B). The difference was statistically significant (CNE-2Z: 35 ± 3 vs 133 ± 7 of shControl, P < 0.01, Figure 4C; 5-8F cell: 75 ± 8 vs 268 ± 2 of shControl, P < 0.01, Figure 4D).

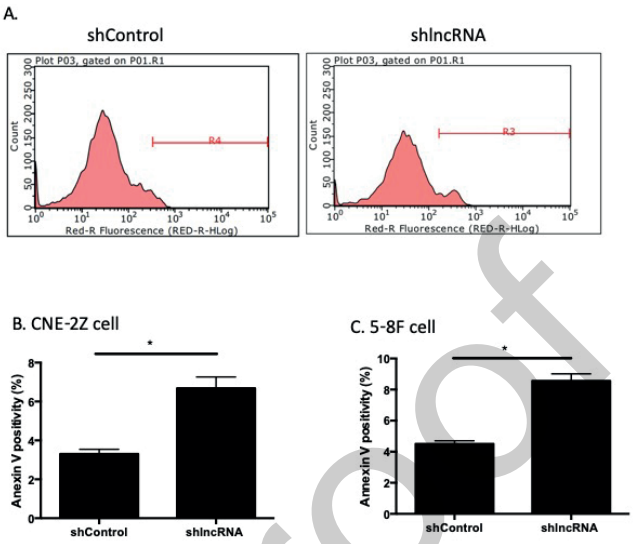

Figure 2. Annexin V expression assessment by flow cytometry. A) Representative histogram of the flow cytometry of assessing Annexin V expression. B and C) Quantitative comparison of Annexin V expression in the CNE-2Z (B) and 5-8F (C) cells transfected with shControl or shlncRNA. Vertical axes: Annexin V positivity (%); horizontal axes: cells transfected with shControl or shlncRNA. * P < 0.05.

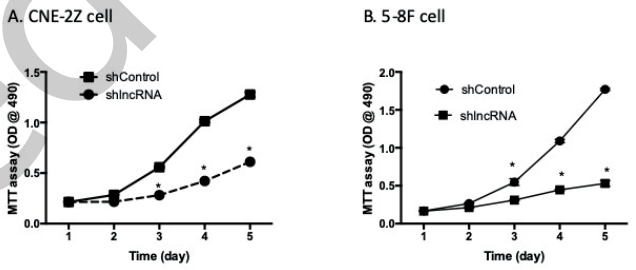

Figure 3. MTT assay. A) CNE-2Z cell line. B) 5-8F cell line. Vertical axes: OD value at 490nm; horizontal axes: time (day). * P < 0.05 compared to shControl cells at corresponding time point.

To further confirm the effect of suppression of lncRNA on tumor formation, subcutaneous xenograft tumor formation was performed in nude mice. As shown in Figure 5, suppression of lncRNA-ENST00000412010 in the CNE-2Z cells reduced the size of nasopharyngeal tumor xenograft, but did not abolish the growth of the tumor (Figure 5A and 5B). Tumor size varied significantly, which led to no significant differences in tumor size between the two groups (Figure 5C) up to day 45 (802.25 ± 356.14 vs 1360.81 ± 647.17 mm3 of shControl). Average of tumor weight on day 45, however, was significantly less in the group of animals injected with the cells lack of lncRNA compared to that of animals injected with the control cells (0.823 ± 0.341 g of the animals injected with the cells lack of lncRNA versus 1.315 ± 0.596 g of the control group, P < 0.05).

Suppression of lncRNA-ENST00000412010 gene resulted

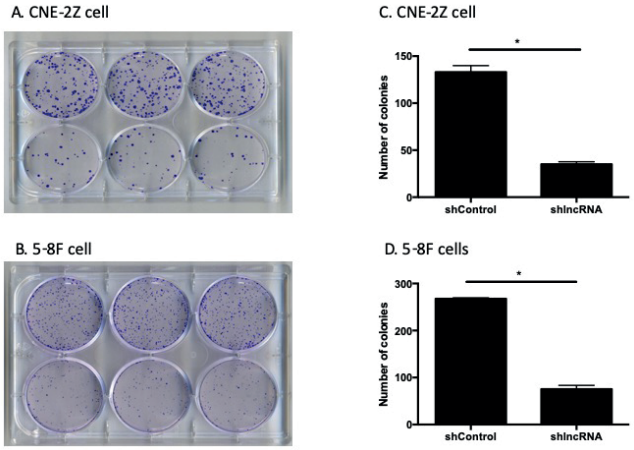

Figure 4. Colony formation assay. A and B) Representative image of colony formation of CNE-2Z (A) and 5-8F (B) cell lines. C and D) Quantitative comparison of the colonies between the cells transfected with shControl or shlncRNA in CNE-2Z (C) and 5-8F (D) cell lines. Vertical axes: Number of colonies; horizontal axes: cells transfected with shControl or shlncRNA. *P < 0.05.

in alteration of genes related with cell growth and inflammation

To explore mechanisms of lncRNA-ENST00000412010 effect on cell survival, gene expression array was performed (data not presented) and alteration of the genes was further confirmed by real time RT-PCR in the CNE-2Z cells. It was found that many genes were altered in the cells lacking lncRNA-ENST00000412010 including genes associated with cell survival, inflammation, and cell signaling. Of them, expression of DNA damage-inducible transcript 3 (DDIT3, 2.72 ± 0.16 fold of control, Figure 6A), dual specificity protein phosphatase 5 (DUSP5, 11.87 ± 0.95 fold of control, Figure 6B), insulin receptor substrate 1 (IRS1, 2.70 ± 0.25 fold of control, Figure 6C), interleukin-6 (IL-6, 2.81 ± 0.33 fold of control, Figure 6D), and tribbles homolog 3 (TRIB3, 2.18 ± 0.21 fold of control, Figure 6E) genes was significantly up-regulated in the cells lacking lncRNA-ENST00000412010, while gene expression of matrix metalloproteinase-7 (MMP-7, 0.24 ± 0.02 fold of control, Figure 6F) and cyclin-dependent kinase 4 inhibitor B (CDKN2B, 0.57 ± 0.01 fold of control, Figure 6 G) was significantly

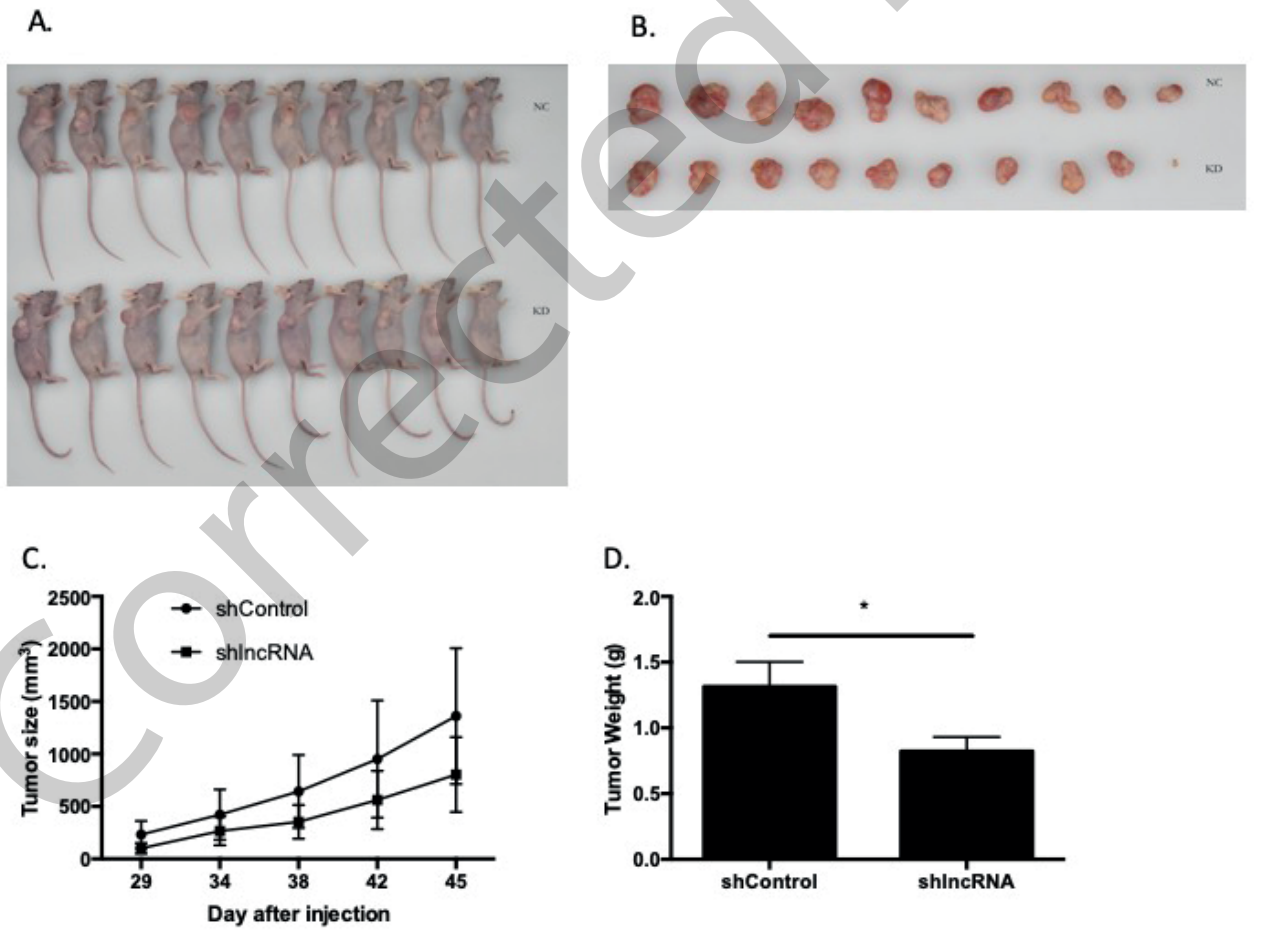

Figure 5. Subcutaneous xenograft tumor formation assay. A) Image of subcutaneous xenograft tumors formed in the nude mice. B) Image of xenograft tumors. C) Comparison of the xenograft tumor size between the cells transfected with shControl or shlncRNA as function of time. Vertical axis: tumor size (mm3); horizontal axis: time (day). D) Average of the xenograft tumor weight between the cells transfected with shControl or shlncRNA. Vertical axis: tumor weight (g); horizontal axis: cells transfected with shControl or shlncRNA. *P < 0.05.

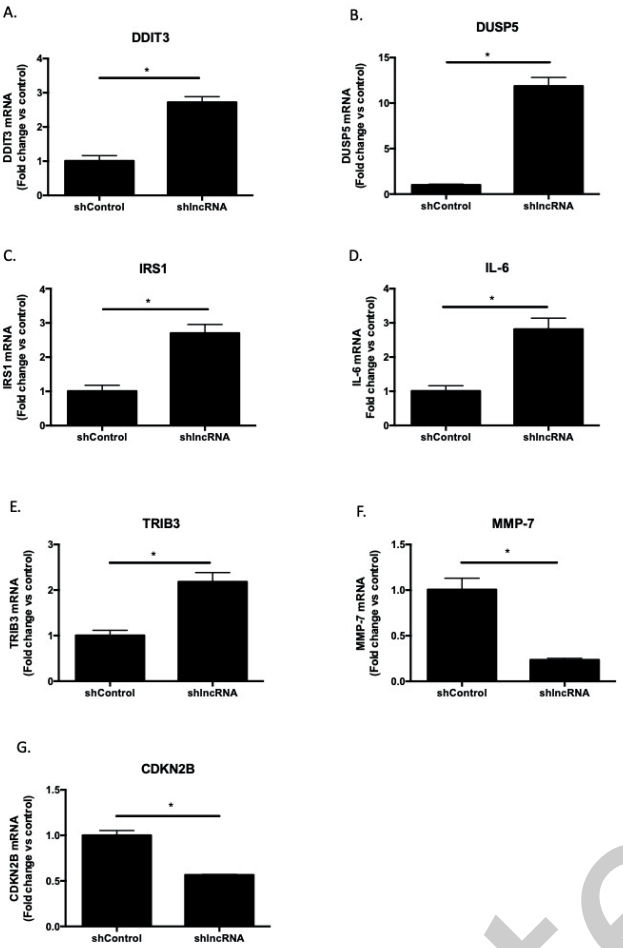

Figure 6. Quantification of the gene expression by real time RT-PCR. A) DNA damage-inducible transcript 3 (DDIT3); B) Dual specificity protein phosphatase 5 (DUSP5); C) Insulin receptor substrate 1 (IRS1); D) Interleukin-6 (IL-6); E) Tribbles homolog 3 (TRIB3); F) matrix metalloproteinase-7 (MMP-7); G) Cyclin-dependent kinase 4 inhibitor B (CDKN2B). Vertical axes: mRNA expression (fold change vs control); horizontal axes: cells transfected with shControl or shlncRNA. *$P < 0.05$.

down-regulated in the cells lacking lncRNA.

Immunoblotting assay further confirmed protein level alteration of the genes. Of the up-regulated genes, however, only DUSP5 protein was significantly increased while DDIT3, IRS1, and TRIB3 proteins were slightly but not significantly changed in the cells lacking lncRNA (Figure 7). In contrast to the mRNA level, IL-6 protein in the cell lysate was significantly lower in the cells lacking lncRNA compared to that in the control cells (Figure 7). Consistent with mRNA levels, proteins of MMP-7 and CDKN2B were significantly lower in the cells lacking lncRNA than that of the control cells (Figure 7).

Discussion

Nasopharyngeal carcinoma (NPC) is exceedingly predominant in Asia, particularly Southern China among Cantonese-speaking Chinese [21]. While NPC incidence has declined gradually [1], pathogenesis of NPC remains to be addressed. Here, we investigated role of lncRNA-ENST00000412010 in regulating NPC cell proliferation, survival, colony formation, and xenograft tumor formation. We found that suppression of lncRNA-ENST00000412010 by shRNA resulted in slower growth, less survival, but higher rate of apoptosis in two different NPC cell lines. Furthermore, NPCs lack of lncRNA-ENST00000412010 nearly lost the ability of forming colonies in vitro and significantly reduced in vivo xenograft tumor formation capability. In addition, both mRNA and protein levels of DUSP5 were significantly increased, while MMP-7 and CDKN2B were significantly decreased in the NPCs transfected with shRNA against lncRNA-ENST00000412010 compared to the NPCs transfected with scramble shRNA. These findings suggested that lncRNA-ENST00000412010 play a role in the tumorigenesis of NPC through modulating cell survival and proliferation.

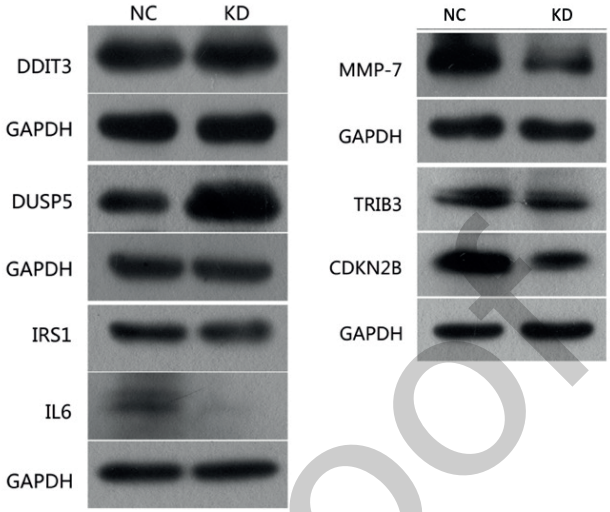

Figure 7. Immunoblotting assay. Following transfection of shControl or shlncRNA, protein levels of the genes potentially targeted by lncRNA in the CNE-2Z cells was assessed by immunoblotting as described in the methods. NC: cells transfected with shControl; KD: cells transfected with shlncRNA. Data presented were one representative image of immunoblotting assays at least performed three time.

Long non-coding RNAs (lncRNAs) are a large and diverse class of transcribed RNA molecules with a length of more than 200 nucleotides that do not encode proteins. Increasing evidences demonstrated that lncRNAs involve in modulating gene expression in various ways including chromatin modification, transcription, and post-transcription [22]. In this content, it has been reported that several lncRNAs are related with NPC pathogenesis. For instance, lncRNA-LINC00460 facilitated NPC tumorigenesis through sponging miR-149-5p to up-regulate IL-6 [10], lncRNA FAM226A promoted NPC tumorigenesis and metastasis by acting as ceRNA to sponge miR-590-3p/miR-

1275 and up-regulate ITGB3 [17], lncRNA HOTAIR contributed to the tumorigenesis of NPC via up-regulating FASN [11], lncRNA LINC01133 mediated NPC tumorigenesis by binding to YBX1 [15], lncRNA C22 or f32-1 contributed to the tumorigenesis of NPC [12], lncRNA PXN-AS1-L promoted the malignancy of NPC cells via up-regulation of SAPCD2 [13], and the lncRNA PVT1 regulated NPC cell proliferation via activating the KAT2A acetyltransferase and stabilizing HIF-1α [14]. Consistent with these previous reports, in the current study, we demonstrated that suppression of lncRNA-ENST00000412010 by shRNA rendered the NPC cells grew slower, underwent apoptosis, and formed less colonies. In addition, shRNA targeting lncRNA-ENST00000412010 decreased expression of cyclin-dependent kinase 4 inhibitor B (CDKN2B) but increased expression of dual specificity protein phosphatase 5 (DUSP5), a phosphatase that can inactivate MAPK, suggesting lncRNA-ENST00000412010 may play a role in modulating NPC proliferation and survival through regulating molecules associated with cell cycle and MAPK signaling.

Xenograft tumor model is often used to test tumorigenesis of human cancer cells. In this study, we used nude mice to examine the role of lncRNA-ENST00000412010 in the development of xenograft tumor formed by a nasopharyngeal carcinoma cell line. We found that average tumor size and weight were significantly smaller or less in the mice injected with NPCs lack of lncRNA-ENST00000412010 in comparison to the mice injected with control NPCs, suggesting lncRNA-ENST00000412010 might contribute to the development of tumor in vivo.

While the effect of lncRNA-ENST00000412010 on specific intracellular signal transduction pathways was not investigated in the current study, effect of lncRNA-ENST00000412010 on molecules associated with cell proliferation, inflammation, and protein kinases or phosphatases was explored following gene expression array analysis on the cells lack of lncRNA-ENST00000412010. It was found that mRNA expression of DNA damage-inducible transcript 3 (DDIT3), dual specificity protein phosphatase 5 (DUSP5, a phosphatase that can inactivate MAPK), insulin receptor substrate 1 (IRS1), interleukin-6 (IL-6), and tribbles homolog 3 (TRIB3, a negative regulator of NF-kB signal pathway) was significantly up-regulated in the cells lacking lncRNA-ENST00000412010. In contrast, the mRNA expression of matrix metalloproteinase-7 (MMP-7) and cyclin-dependent kinase 4 inhibitor B (CDKN2B) was significantly down-regulated in the cells lacking lncRNA-ENST00000412010. Consistent with direction of mRNA expression, protein levels of MMP-7 and CDKN2B were significantly reduced in the cells lacking lncRNA-ENST00000412010, suggesting CDKN2B and MMP-7 might be associated with the cell survival, proliferation, and migration of NPCs through regulating cell cycle and matrix metalloproteinases. Interestingly, of the molecules with mRNA up-regulation, only DUSP5 protein was increased in the same direction as the mRNA was in the cells lacking lncRNA-ENST00000412010. In case of intracellular IL-6 protein determined by immunoblotting, however, in contrast to mRNA expression level, it was significantly decreased in the cells lacking lncRNA-ENST00000412010, while proteins of DDIT3, TRIB3, and IRS1 were not significantly different between the NPCs lacking lncRNA-ENST00000412010 and control cells. While the mechanisms of inconsistency in the expressions of mRNA and intracellular protein in the aforementioned molecules remain to be defined, possibly increased extracellular release of the molecules, especially in case of IL-6, could partially explain the discrepancy, which remains to be determined.

Previous studies on the cancer pathogenesis have been focused on protein coding genes. Recently, accumulating studies have reported that lncRNAs function as biomarkers, oncogenes, or tumor suppressors in variety kinds of cancers[23]. For instance, lncRNAs have been reported to play important roles in cell cycle [24], apoptosis [25], and regulation of signal transduction pathways [26, 27]. However, while the gene expression array of lncRNAs in oncology is growing, its implication for patient treatment remains under-explored [28]. The majority of lncRNAs reported so far are derived from investigations on particular cancer types. For instance, SChLAP1 is identified as a specific lncRNA in prostate cancer[29]. Considering that malignant tumors may share common pathogenic pathways, the current study investigated potential role of lncRNA-ENST00000412010 in nasopharyngeal cancer cell lines and found that lncRNA-ENST00000412010 may function as an oncogene in the NPCs. Findings of the current study suggested that lncRNA-ENST00000412010 could be a therapeutic target for nasopharyngeal cancer patients through structural silencing RNAi technology or functional blocking molecular inhibitor. However, clinical implication of lncRNA-ENST00000412010 in NPC diagnosis and treatment remains to be further investigated.

Despite findings of the association of lncRNA-ENST00000412010 with NPC, there were limitations in the current study. First, how the lncRNA-ENST00000412010 drives NPC tumorigenesis remains to be further investigated. Second, aberrant expression of lncRNA-ENST00000412010 and its potential role as biomarker or oncogenic driver in NPC patients remains to be confirmed. A comparison of lncRNA-ENST00000412010 expression in the tumor tissues from NPC patients and normal tissues could be the next step of investigation. Third, while the current study demonstrated that suppression of lncRNA-ENST00000412010 in the NPC cell line resulted in inhibition of xenograft tumor formation, its potential application on patients is more limited as it involves targeted genetic manipulation[30, 31].

Conclusion

The current study demonstrated that, compared to the NPCs transfected with shControl, NPCs transfected with shRNA targeting lncRNA-ENST00000412010 grew slower with higher

percentage of apoptosis but lower capacity of colony formation or xenograft tumor formation. Matrix metalloproteinase-7 (MMP-7) and cyclin-dependent kinase 4 inhibitor B (CDKN2BM) were significantly down-regulated, but DUSP5 was significantly up-regulated in the NPCs lacking lncRNA-ENST00000412010, suggesting lncRNA-ENST00000412010 modulate NPC proliferation and survival through regulating molecules associated with signal transduction of cell growth and inflammation. Clinical application of lncRNA-ENST00000412010 as a therapeutic target for NPC, however, remains to be further investigated.

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Authorship contribution

YC and JPF contributed to the conception and design of the study; HP contributed to the acquisition of data; XQL and CQL performed the experiments; HHL contributed to the analysis of date and wrote the manuscript; All authors reviewed and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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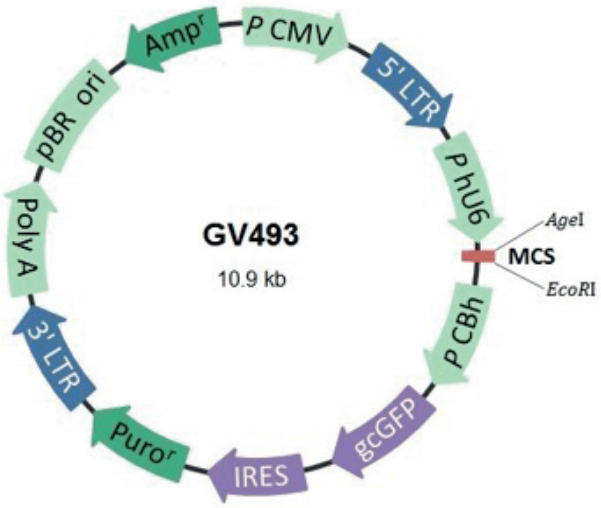
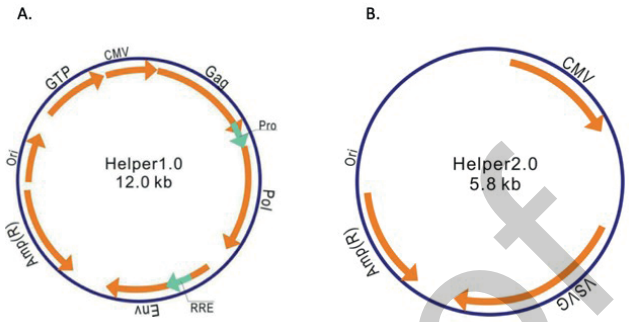
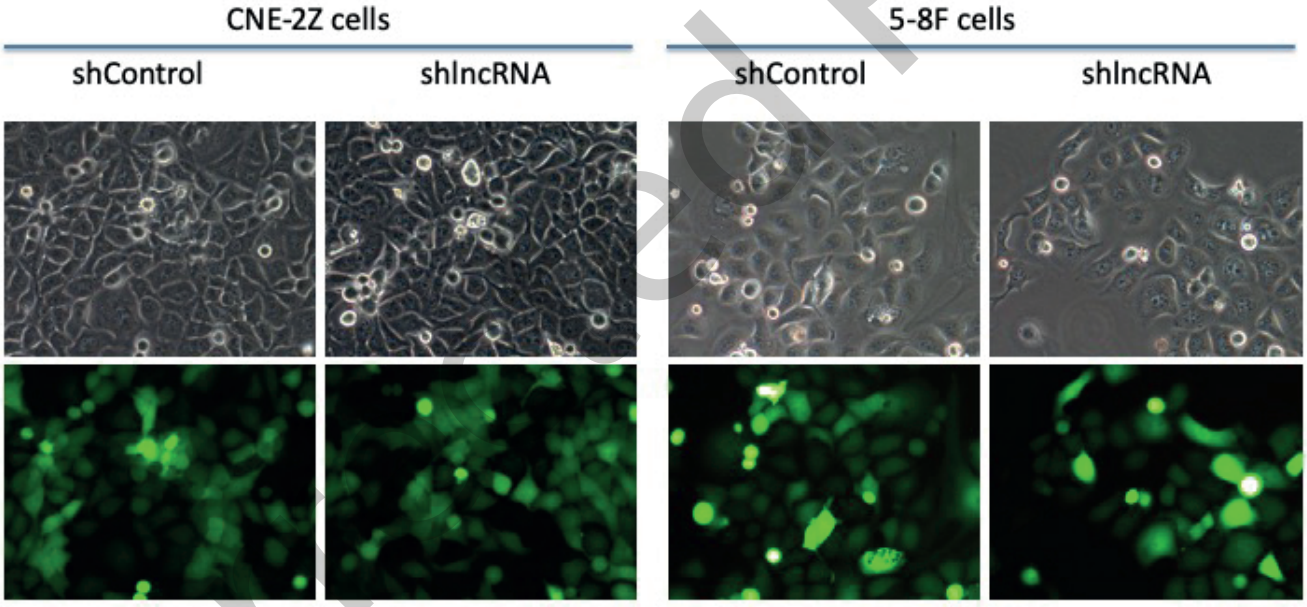
SUPPLEMENTARY DATA



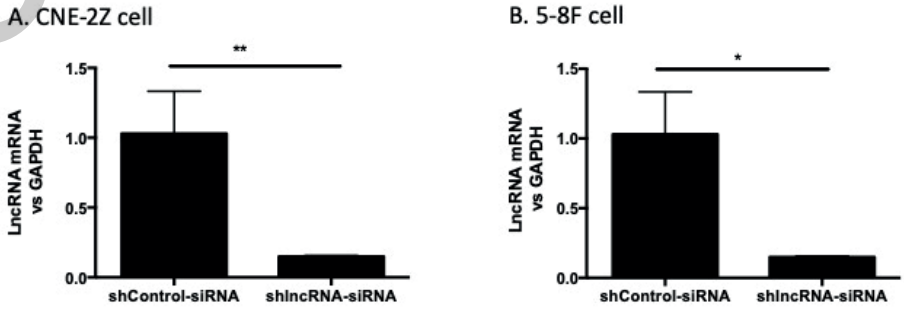
Supplement Figure 1. Structure of the GV493 vector.



Supplement Figure 2. Structure of pHelper 1.0 vector (A) and pHelper 2.0 vector (B).



Supplement Figure 3. Representative image of the CNE-2Z and 5-8F cells following transfection of shControl or shlncRNA plasmid. Magnification: x 200.



Supplement Figure 4. Quantification of lncRNA-ENST00000412010 by real time RT-PCR in the cells transfected with shControl or shlncRNA-ENST00000412010. A) CNE-2Z cells. B) 5-8F cells. Vertical axes: LncRNA-ENST00000412010 mRNA expression versus GAPDH; horizontal axes: cells transfected with shControl or shlncRNA.