Long noncoding RNA DRAIC acts as a microRNA-122 sponge to facilitate nasopharyngeal carcinoma cell proliferation, migration and invasion via regulating SATB1

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ABSTRACT
Increasing evidences have revealed that long noncoding RNAs (lncRNAs) are frequently involved in various cancers. However, the expression and function of lncRNA DRAIC in nasopharyngeal carcinoma (NPC) remain unknown. In this study, we found that DRAIC was significantly increased in NPC tissues. Increased expression of DRAIC was positively correlated with advanced clinical stages of NPC patients. Functional assays revealed that ectopic expression of DRAIC enhances NPC cell growth, migration and invasion. DRAIC knockdown represses NPC cell growth, migration and invasion. Mechanistically, we identified two miR-122 binding sites on DRAIC. RNA pull-down, RNA immunoprecipitation, and dual-luciferase reporter assays confirmed the binding of DRAIC to miR-122. Via binding of miR-122, DRAIC upregulated the expression of miR-122 target SATB1, which was abolished by the mutation of miR-122 binding sites on SATB1. Moreover, the oncogenic roles of DRAIC on NPC were reversed by the mutation of miR-122 binding sites on SATB1, simultaneous overexpression of miR-122, or depletion of SATB1. In addition, the expression of SATB1 was also increased and positively associated with that of DRAIC in NPC tissues. In conclusion, these findings revealed the important roles of DRAIC-miR-122-SATB1 axis in NPC and suggested that DRAIC may be a potential therapeutic target for NPC.

Introduction
Nasopharyngeal carcinoma (NPC) is an epithelial malignancy which originates from the nasopharynx epithelium [1,2]. There are 129,079 estimated new NPC cases and 72,987 estimated deaths of NPC in 2018 worldwide [3]. Intensity-modulated radiotherapy combined with chemotherapy is the standard therapy for NPC [2]. Although the prognoses of most early stages NPC are relative good under standard therapy, the outcomes of NPC patients diagnosed at late stages are still frustrating, with a median survival about only 1 year [4]. Therefore, enhancing the understanding of the molecular mechanisms underlying NPC progression is critically important for the development of more efficient treatment for NPC.

Accumulating researches have identified many genetic and epigenetic aberrations in NPC, such as the mutation of ARID1A, TP53, and PIN3CA, dysregulated DNA methylation and histone methylation, and others [5–7]. Intriguingly, recent advances have shown that many regulator RNAs are dysregulated and play critical roles in tumors initiation and progression [8]. According to the length of regulator RNAs, there are mainly two class of regulator RNAs. Long noncoding RNAs (lncRNAs) are a class of RNAs with more than 200 nucleotides in length and lacking protein-coding capacity [9,10]. Transcriptome sequencings have found many aberrantly expressed lncRNAs in numbers diseases, including cancers [11–13]. In addition, lncRNAs are shown to have complex and various roles in a variety of pathophysiological processes [14–17]. As to cancers, lncRNAs are shown to regulate almost all malignant behaviours of cancers, such as proliferation, apoptosis, migration, invasion, stem cell pluripotency, drug-resistance, and so on [18–21]. The molecular mechanisms mediating the roles of lncRNAs are various [22,23]. lncRNAs could interact with epigenetic enzymes, transcription factors, and/or even directly with DNA to regulate DNA replication, chromatin structure, and transcription [24,25]. lncRNAs could also directly interact with other miRNAs, change mRNA splicing, stability, and translation [21]. In addition, lncRNAs could directly bind microRNAs (miRNAs) and relieve the repressive roles of miRNAs on their targets [26].

miRNAs are another class of regulatory RNAs with 19–25 nucleotides in length [27]. Similar to lncRNAs, miRNAs are also frequently dysregulated in numbers diseases, including cancers [28]. Similarly, miRNAs are also reported to play important roles in various pathophysiological processes [29–34]. As to caners, some miRNAs are revealed to have oncogenic roles via targeting tumor suppressors, and other
miRNAs are demonstrated to have tumor-suppressive roles via targeting oncogenes [35–38]. In our previous report, we found that miR-122 suppressed NPC cell proliferation, migration and invasion via targeting SATB1 [39].

Many lncRNAs are shown to function as competing endogenous RNAs (ceRNAs) to competitively bind miRNAs and reverse the roles of miRNAs in cancers [40,41]. Therefore, we searched the lncRNAs which could competitively bind miR-122 and reverse the tumor-suppressive roles of miR-122 in NPC. In this study, we found that lncRNA DRAIC could specifically bind miR-122. We further investigated the expression, clinical significances, biological roles, and detailed molecular mechanisms of DRAIC in NPC.

### Materials and methods

#### Cell culture

NPC cell lines CNE-1 and C666-1 were purchased from Gaining Biological (Shanghai, China) and cultured in RPMI-1640 medium (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) added with 10% fetal bovine serum (FBS, Invitrogen). The cells were maintained at 37°C humidified incubator containing 5% CO₂.

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

Total RNA was extracted using Trizol reagent (Invitrogen) following the provided protocol. Next, extracted RNA was reverse transcribed into complementary DNA (cDNA) using cDNA Synthesis kit (Thermo Fisher Scientific). qRT-PCR was performed at ABI 7500 (Applied Biosystems, Thermo Fisher Scientific) using SYBR Green Mix (Takara, Dalian, Liaoning, China). The primers sequences were as follows: DRAIC: (F) 5’-GCAAAGACTGCGCCCTCCTA-3’, (R) 5’-CAGCTGCCACCATTTGGA-3’; SATB1: (F) 5’-CTGAAAGGATGAAACCC-3’, (R) 5’-GAGGAA GACTGAGAAC-3’; GAPDH: (F) 5’-GCTTCCCTCTGACCTCAACA-3’, (R) 5’-GTGAGGGTCTCCTCTCTTCC-3’. GAPDH was employed as internal control for the quantitation of lncRNAs and mRNAs expression. For the quantitation of miRNAs expression, qRT-PCR was performed at ABI 7500 using TaqMan microRNA assays following the provided protocol. U6 was employed as an internal control for the quantitation of miRNAs expression. Relative expression levels were determined using the 2⁻ΔΔCt method.

#### Isolation of cytoplasmic and nuclear RNA

Cytoplasmic and nuclear RNA were isolated and purified using the PARIS Kit (Ambion, Thermo Fisher Scientific) following the provided protocol. The relative expression of lncRNAs in cytoplasm and nucleus was determined using qRT-PCR as above described.

### Vectors construction and transfection

The cDNA encoding DRAIC was PCR-amplified using Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher Scientific) with the primers (F) 5’-GGGGTACCAAGGGAGCTGGTCCAGGA-3’ and (R) 5’-GCTCTAGACAGAGACGGAGAGATTTATTT-3’. The PCR products were subcloned into the Kpn I and Xba I sites of pcDNA3.1(+) vector (Invitrogen) to construct DRAIC overexpression vector pcDNA-DRAIC. The PCR products were also subcloned into the Kpn I and Xba I sites of pSPT19 vector (Roche, Mannheim, Germany) to construct DRAIC in vitro transcription vector pSPT19-DRAIC. The cDNA encoding miR-122 binding sites mutated DRAIC was synthesized by GenScript (Nanjing, China) and subcloned into the Kpn I and Xba I sites of pcDNA3.1(+) vector to construct miR-122 binding sites mutated DRAIC overexpression vector pcDNA-DRAIC-mut.

The cDNA encoding miR-122 binding sites mutated DRAIC was also subcloned into the Kpn I and Xba I sites of pSPT19 vector to construct miR-122 binding sites mutated DRAIC in vitro transcription vector pSPT19-DRAIC-mut. Two independent cDNA oligonucleotides suppressing DRAIC expression were synthesized and inserted to the SuperSilencing shRNA expression vector pGPU6/Neo (GenePharma, Shanghai, China). The target sites were as follows: sh-DRAIC-1, 5’-GCCUCACACAGAAAGCAAA-3’; sh-DRAIC-2, 5’-GCAGAG UAUGAACAUAUA-3’ [42]. SATB1 specific shRNA was purchased from FulenGen (Guangzhou, China). DRAIC sequences containing the miR-122 binding sites were PCR-amplified from pcDNA-DRAIC using Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix with the primers (F) 5’-CGAGGCTTGAATCACCTGTCCTACGATGAGGGAAAGC-3’ and (R) 5’-GCTTCTAGACCTGAGTTAGATGGGTGGAAC-3’. The PCR products were subcloned into the Sac I and Xba I sites of pmirGLO vector (Promega, Madison, WI, USA) to construct luciferase reporter vector pmirGLO-DRAIC. pmirGLO-DRAIC-mut was constructed as above described from pcDNA-DRAIC-mut. SATB1 3’UTR was PCR-amplified using Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix with the primers (F) 5’-CGAGGCTTGAATCACCTGTCCTACGATGAGGGAAAGC-3’ and (R) 5’-GCTTCTAGACCTGAGTTAGATGGGTGGAAC-3’. The PCR products were subcloned into the Sac I and Xba I sites of pmirGLO vector to construct luciferase reporter vector pmirGLO-SATB1.

miR-122 mimics, inhibitors, and their respective negative control (NC) were purchased from RiboBio (Guangzhou, China). Transfection was conducted using Lipofectamine 3000 (Invitrogen) following the provided protocol. After 48 h of transfection, cells were harvested for further experiments.

### Stable cell lines construction

To construct wild type or miR-122 binding sites mutated DRAIC stably overexpressed NPC cells, pcDNA-DRAIC or pcDNA-DRAIC-mut was transfected into CNE-1 and C666-1 cells. After 48 h of transfection, cells were selected with neomycin. To construct DRAIC stably depleted NPC cells, DRAIC specific shRNAs were transfected into CNE-1 and C666-1 cells. After 48 h of transfection, cells were selected with neomycin. To construct miR-122 and DRAIC concurrently stably
overexpressed NPC cells, recombinant lentiviruses containing pre-hsa-miR-122 were purchased from FulenGen. DRAIC stably overexpressed CNE-1 and C666-1 cells were transfected with miR-122 overexpression lentiviruses and selected with neomycin and puromycin. To construct DRAIC stably overexpressed and concurrently SATB1 stably depleted NPC cells, DRAIC stably overexpressed CNE-1 and C666-1 cells were transfected with SATB1 specific shRNAs and selected with neomycin and puromycin. The overexpression and knockdown efficiencies were detected by qRT-PCR and Western blot.

**Dual-luciferase reporter assay**

pmirGLO, pmirGLO-DRAIC, or pmirGLO-DRAIC-mut was cotransfected with miR-122 mimics or miR-122 inhibitors into CNE-1 cells. pmirGLO or pmirGLO-SATB1 was cotransfected with pcDNA, pcDNA-DRAIC, pcDNA-DRAIC-mut, sh-NC, sh-DRAIC-1, or sh-DRAIC-2 into CNE-1 cells. After 48 h of transfection, the luciferase activity was detected using the Dual-Luciferase Reporter Assay System (Promega) following the provided protocol, and normalized to Renilla luciferase activity.

**RNA immunoprecipitation (RIP) assay**

miR-122 mimics or miR-122 inhibitors were transfected into CNE-1 cells. After 48 h of transfection, RIP assays were conducted using an anti-AGO2 antibody (5 mg per reaction; 03–110, Millipore, Bedford, MA, USA) and the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore) following the provided protocol.

**RNA pull-down assay**

Wild type DRAIC or miR-122 binding sites mutated DRAIC was *in vitro* transcribed respectively from the vector psiP19-DRAIC or psiP19-DRAIC-mut, and biotin-labelled using the Biotin RNA Labeling Mix (Roche) and Sp6 RNA polymerase (Roche). The *in vitro* transcribed transcripts were treated with RNase-free DNase I (Roche), and purified using the RNeasy Mini Kit (Qiagen) following the provided protocols. Three mg of purified biotin-labelled transcripts were incubated with 1 mg of whole-cell lysates from CNE-1 cells for 1 h at 25 °C. Next, the complexes were extracted using streptavidin agarose beads (Invitrogen). The RNA present in the pull-down material was determined using qRT-PCR as above described.

**Western blot**

Total protein was extracted from indicated NPC cells using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Haimen, Jiangsu, China) following the provided protocol. Protein concentration was detected by the bicinchoninic acid (BCA) protein determination kit (Beyotime). Subsequently, an equal amount of protein was separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by being transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore). The membranes were incubated with 5% fat-free milk at room temperature for 2 h and then incubated with primary antibodies (SATB1: ab109122, GAPDH: ab181602; Abcam, Cambridge, MA, USA) overnight at 4 °C. Next, the membranes were washed three times using Tris-buffered saline with Tween-20 and incubated with horseradish-peroxidase conjugated secondary antibody (ab6721, Abcam). Blots were visualized by the enhanced chemiluminescence reagent.

**Cell proliferation assay**

Cell counting kit-8 (CCK-8) assays and 5-ethyl-2-deoxyuridine (EdU) incorporation assays were conducted to evaluate cell proliferation. For CCK-8 assays, indicted NPC cells were plated in 96-well plate at the density of 3 × 10^3 cells per well. Subsequently, 10 μl CCK-8 solutions (Dojindo Laboratories, Kumamoto, Japan) were added to each well and incubated for another 1 h at 37 °C. The optical density (OD) at 450 nm was detected to indicate cell proliferation rate by a microplate reader. EdU incorporation assays were conducted using the EdU Kit (RiboBio) following the provided protocol. The results were counted using Zeiss photomicroscope (Carl Zeiss, Oberkochen, Germany).

**Cell migration and invasion assays**

Transwell migration and invasion assays were conducted to evaluate cell migration and invasion, respectively. Transwell invasion assays were carried out using transwell chamber (Corning, New York, NY, USA) with 8 μm pores coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Transwell migration assays were carried out using Transwell chamber (Corning) with 8 μm pores without Matrigel. Indicated NPC cells resuspended in serum-free medium were plated into the upper chamber. The medium containing 20% FBS was plated into the lower chamber. After incubation for 48 h, cells remaining on the upper side were removed. The migrated or invasive cells on the lower side of the transwell chamber were fixed in 10% methanol, stained using 0.1% crystal violet for 15 min at room temperature, and counted using Zeiss photomicroscope.

**Tissue samples**

A total of 32 NPC biopsy tissues and 20 normal nasopharyngeal epithelial tissues were collected at the Second Affiliated Hospital of Nanchang University (Nanchang, China). None of the patients received any anti-cancer therapy before the biopsy. All the patients were staged following the 8th edition of the American Joint Committee on Cancer (AJCC) staging manual. All tissues samples were confirmed by histopathological diagnosis. This study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Nanchang University (Nanchang, China). Written informed consents were acquired from all patients.
**Statistical analysis**

Statistical analyses were performed using GraphPad Prism 6 Software (La Jolla, CA, USA). Data were presented as mean ± standard deviation (SD). The differences between two groups were analyzed by Student’s t-test. The differences between the two groups were compared by Student’s t-test. The differences between more than two groups were compared by one-way analysis of variance (ANOVA) with Dunnett’s multiple comparisons test. The differences between the two groups of tissue samples were compared by Mann Whitney test. The correlation between genes’ expression in tissue samples was analyzed by Pearson’s correlation analysis. p < .05 was considered as statistically significant.

**Results**

**DRAIC specifically sponges miR-122**

We searched the lncRNAs which have potential miR-122 binding sites using the StarBase 2.0 and TargetScan prediction algorithm [43,44]. Among the predicted lncRNAs, we noted lncRNA DRAIC, which has two potential miR-122 binding sites (Figure 1(A)). Subcellular location analyzing revealed that DRAIC was mainly located in the cytoplasm (Figure 1(B)), which supports the potential binding between DRAIC and miR-122. To investigate whether DRAIC binds to miR-122, RNA pull-down assays were performed using in vitro transcribed DRAIC. As shown in Figure 1(C), miR-122 was significantly enriched in DRAIC group. Mutation of the two predicted miR-122 binding sites on DRAIC abrogated the enrichment of miR-122 (Figure 1(C)). DRAIC sequences containing the predicted miR-122 binding sites were subcloned into the pmirGLO vector and located in the downstream of luciferase reporter gene. Dual-luciferase reporter assays demonstrated that miR-122 mimics markedly repressed the luciferase activity of the constructed reporter containing DRAIC (Figure 1(D)). The mutation of the miR-122 binding sites abolished the repressive roles of miR-122 on luciferase activity (Figure 1(D)). Dual-luciferase reporter assays also demonstrated that miR-122 inhibitors markedly increased the luciferase activity of the constructed reporter containing DRAIC, which was abrogated by the mutation of the miR-122 binding sites on DRAIC (Figure 1(E)). miRNAs were known to bind AGO2 and further together bind their targets [40]. Therefore, RIP assays with AGO2 specific antibody were performed. As shown in Figure 1(F), miR-122 mimics significantly promoted the binding between AGO2 and DRAIC. miR-122 inhibitors decreased the binding between AGO2 and DRAIC (Figure 1(F)). Collectively, these data demonstrated that DRAIC specifically binds miR-122.

**DRAIC upregulates SATB1 via sponging miR-122**

In our previous report, we have identified SATB1 as a critical target of miR-122 in NPC [39]. In this study, we further investigated whether DRAIC regulated SATB1 via sponging miR-122. SATB1 3’UTR containing miR-122 targeting sites were subcloned into the pmirGLO vector and located in the downstream of luciferase reporter gene. Dual-luciferase reporter assays demonstrated that DRAIC overexpression increased the luciferase activity of the constructed reporter containing SATB1 3’UTR (Figure 2(A)). Overexpression of miR-122 binding sites mutated DRAIC did not change the luciferase activity of the constructed reporter containing SATB1 3’UTR (Figure 2(A)). Dual-luciferase reporter assays also demonstrated that DRAIC knockdown decreased the luciferase activity of the constructed reporter containing SATB1 3’UTR (Figure 2(B)). qRT-PCR assays showed that DRAIC overexpression increased SATB1 mRNA level, which was abolished by the mutation of miR-122 binding sites on DRAIC (Figure 2(C)). qRT-PCR assays also showed that DRAIC knockdown decreased SATB1 mRNA level (Figure 2(D)). Western blot assays showed that DRAIC overexpression increased SATB1 protein level, which was abolished by the mutation of miR-122 binding sites on DRAIC (Figure 2(E)). Western blot assays also showed that DRAIC knockdown decreased SATB1 protein level (Figure 2(F)). Collectively, these data demonstrated that DRAIC upregulates SATB1 in a miR-122 dependent manner.

**DRAIC overexpression enhances NPC cell growth, migration and invasion**

Next, we investigated the biological roles of DRAIC in NPC. DRAIC stably overexpressed CNE-1 and C666-1 cells were constructed via stable transfection of DRAIC overexpression vector (Figure 3(A,B)). miR-122 binding sites mutated DRAIC was also stably overexpressed in CNE-1 and C666-1 cells with equal overexpression efficiency compared with wild type DRAIC (Figure 3(A,B)). CCK-8 assay revealed that DRAIC overexpression significantly enhanced CNE-1 and C666-1 cell growth, which was abrogated by the mutation of miR-122 binding sites (Figure 3(C,D)). To validate the roles of DRAIC on NPC cell growth, EdU incorporation assays were conducted. As shown in Figure 3(E), CNE-1 and C666-1 cells with DRAIC overexpression displayed much more EdU positive cells. The mutation of miR-122 binding sites abrogated the increasing of the number of EdU positive cells (Figure 3(E)). Additionally, transwell migration assays revealed that the migrated cell numbers of DRAIC stably overexpressed CNE-1 and C666-1 cells were much more than those of control cells (Figure 3(F)). Transwell migration assays revealed that the invasive cell numbers of DRAIC stably overexpressed CNE-1 and C666-1 cells were also much more than those of control cells (Figure 3(G)). Collectively, these data demonstrated that DRAIC overexpression enhances NPC cell growth, migration and invasion.

**DRAIC knockdown inhibits NPC cell growth, migration and invasion**

To further validate the biological roles of DRAIC in NPC, DRAIC stably depleted CNE-1 and C666-1 cells were constructed via stable transfection of DRAIC specific shRNAs (Figure 4(A,B)). CCK-8 assay revealed that DRAIC knockdown significantly inhibited CNE-1 and C666-1 cell growth (Figure 4(C,D)). EdU incorporation assays further showed that CNE-1...
and C666-1 cells with DRAIC knockdown displayed much less EdU positive cells (Figure 4(E)). Furthermore, transwell migration assays revealed that the migrated cell numbers of DRAIC stably depleted CNE-1 and C666-1 cells were much less than those of control cells (Figure 4(F)). Transwell invasion assays revealed that the invasive cell numbers of DRAIC stably depleted CNE-1 and C666-1 cells were also much less than those of control cells (Figure 4(G)). Collectively, these data demonstrated that DRAIC knockdown inhibits NPC cell growth, migration and invasion.

**MiR-122 overexpression abrogates the roles of DRAIC overexpression on NPC cell growth, migration and invasion**

To investigate whether the oncogenic roles of DRAIC in NPC were dependent on the sponging of miR-122, we stably overexpressed miR-122 in DRAIC stably overexpressed CNE-1 and C666-1 cells via infection of miR-122 overexpression lentivirus. CCK-8 assay revealed that miR-122 overexpression reversed the increased cell proliferation rate caused by DRAIC
overexpression (Figure 5(A,B)). EdU incorporation assays also revealed that miR-122 overexpression reversed the increased number of EdU positive cells caused by DRAIC overexpression (Figure 5(C)). Additionally, transwell migration assays revealed that miR-122 overexpression reversed the increased number of migrated cells caused by DRAIC overexpression (Figure 5(D)). Transwell invasion assays revealed that miR-122 overexpression reversed the increased number of invasive cells caused by DRAIC overexpression (Figure 5(E)). Collectively, these data demonstrated that DRAIC enhances NPC cell growth, migration and invasion in a miR-122 dependent manner.

**SATB1 knockdown reverses the roles of DRAIC overexpression on NPC cell growth, migration and invasion**

To further investigate whether the oncogenic roles of DRAIC in NPC were dependent on the upregulation of SATB1, we stably depleted SATB1 in DRAIC stably overexpressed CNE-1 and C666-1 cells via stable transfection of SATB1 specific shRNAs (Figure 6(A,B)). CCK-8 assay revealed that SATB1 knockdown reversed the increased cell proliferation rate caused by DRAIC overexpression (Figure 6(C,D)). EdU incorporation assays also revealed that SATB1 knockdown reversed the increased number of EdU positive cells caused by DRAIC overexpression (Figure 6(E)). Additionally, transwell migration assays revealed that SATB1 knockdown reversed the increased number of migrated cells caused by DRAIC overexpression (Figure 6(F)). Transwell invasion assays revealed that SATB1 knockdown reversed the increased number of invasive cells caused by DRAIC overexpression (Figure 6(G)). Collectively, these data demonstrated that DRAIC enhances NPC cell growth, migration and invasion further in a SATB1 dependent manner.

**DRAIC and SATB1 are both upregulated in NPC tissues**

To investigate the clinical significances of DRAIC/miR-122/SATB1 regulation axis in NPC, we measured DRAIC and SATB1 expression in 32 NPC tissues and 20 normal nasopharyngeal epithelial tissues by qRT-PCR. As shown in Figure 7(A), DRAIC expression was significantly increased in NPC tissues compared with normal nasopharyngeal epithelial...
tissues. Analysis of the correlation between DRAIC expression and clinical stages revealed that DRAIC was highly expressed in III–IV stages NPC tissues compared with I–II stages NPC tissues (Figure 7(B)). SATB1 expression was also significantly increased in NPC tissues compared with normal nasopharyngeal epithelial tissues (Figure 7(C)). Analysis of the correlation between SATB1 expression and clinical stages revealed that SATB1 was also highly expressed in III–IV stages NPC tissues.
compared with I–II stages NPC tissues (Figure 7(D)). In addition, DRAIC expression was positively correlated with SATB1 expression in NPC tissues (Figure 7(E)).

Discussion

High throughput transcriptome sequencings have identified more than 58,000 different species of lncRNAs [45]. Nevertheless, the number of protein-coding genes is only about 21,000 [45]. Among these masses of lncRNAs, many may have important roles in cancers. LncRNA DANCR was reported to promote NPC progression via enhancing IL-6/STAT3 signaling [46]. LncRNA PTPRG-AS1 was reported to modulate radiosensitivity and metastasis of NPC cells via sponging miR-194-3p [47]. LncRNA AFAP1-AS1 was reported to facilitate NPC metastasis via modulating Rho/Rac pathway [48]. Although the roles of several lncRNAs in NPC have been identified, the roles of most other lncRNAs in NPC are still unknown.

In our previous study, we have identified miR-122 as a tumor suppressor in NPC [39]. In this study, using
bioinformatics analysis and experimental verifications, we found that IncRNA DRAIC could specifically act as a miR-122 sponge and relieve the repressive roles of miR-122 on SATB1. Via sponging miR-122 and upregulating SATB1, DRAIC facilitates NPC cell growth, migration, and invasion. DRAIC locates at chromosome 15q23, which was reported to be a tumour-suppressive locus in prostate cancer [49]. However, DRAIC was also reported to promote cell proliferation in breast cancer [42]. Another report showed that DRAIC was upregulated in breast cancer tissues and associated with poor prognosis.

Figure 5. Overexpression of miR-122 reversed the roles of DRAIC on NPC cell growth, migration and invasion. (A,B) miR-122 expression in miR-122 and DRAIC concurrently stably overexpressed CNE-1 (A) and C666-1 (B) cells was determined by qRT-PCR. (C,D) Cell proliferation of miR-122 and DRAIC concurrently stably overexpressed CNE-1 (C) and C666-1 (D) cells was determined by CCK-8 assays. (E) Cell proliferation of miR-122 and DRAIC concurrently stably overexpressed CNE-1 and C666-1 cells was determined by EdU incorporation assays. Scale bars, 100 μm. (F) Cell migration of miR-122 and DRAIC concurrently stably overexpressed CNE-1 and C666-1 cells was determined by transwell migration assays. Scale bars, 100 μm. (G) Cell invasion of miR-122 and DRAIC concurrently stably overexpressed CNE-1 and C666-1 cells was determined by transwell invasion assays. Scale bars, 100 μm. Data represent the mean ± SD of three independent experiments. ns: not significant; **p < .01; ***p < .001.
of breast cancer patients [50]. Rajasekaran et al. reported that DRAIC slowed retinoblastoma cell growth [51]. Hence, the expression and roles of DRAIC in different cancers are various and may be dependent on the special context in different cancers. In this study, using gain-of-function and loss-of-function assays we found that ectopic expression of DRAIC facilitates NPC cell growth, migration, and invasion. In contrast, knockdown of DRAIC represses NPC cell growth, migration, and invasion. The same results were acquired in two different NPC cell lines, which further support the oncogenic roles of DRAIC in NPC. Furthermore, we found that the expression of DRAIC was significantly increased in NPC.

Figure 6. SATB1 knockdown abrogated the roles of DRAIC on NPC cell growth, migration and invasion. (A,B) SATB1 protein levels in DRAIC stably overexpressed and concurrently SATB1 stably depleted CNE-1 (A) and C666-1 (B) cells were determined by Western blot. (C,D) Cell proliferation of DRAIC stably overexpressed and concurrently SATB1 stably depleted CNE-1 (C) and C666-1 (D) cells was determined by CCK-8 assays. (E) Cell proliferation of DRAIC stably overexpressed and concurrently SATB1 stably depleted CNE-1 and C666-1 cells was determined by EdU incorporation assays. Scale bars, 100 μm. (F) Cell migration of DRAIC stably overexpressed and concurrently SATB1 stably depleted CNE-1 and C666-1 cells was determined by transwell migration assays. Scale bars, 100 μm. (G) Cell invasion of DRAIC stably overexpressed and concurrently SATB1 stably depleted CNE-1 and C666-1 cells was determined by transwell invasion assays. Scale bars, 100 μm. Data represent the mean ± SD of three independent experiments. ns: not significant; **p < .01; ***p < .001.
tissues compared with that in normal nasopharyngeal epithelial tissues. Increased expression of DRAIC was positively associated with advanced clinical stages of NPC. Thus, these findings suggested that DRAIC is overexpressed and functions as an oncogene in NPC.

One of the important mechanisms of action of lncRNAs is to competitively bind common miRNAs and relieve the repressive roles of miRNAs on their targets [52]. These lncRNAs were also classified as competing endogenous RNAs (ceRNAs) [53]. Via competitively binding miRNAs with oncogenic or tumor suppressive roles, lncRNAs could exert tumor suppressive or oncogenic roles, respectively [40]. LncRNA-ATB was reported to bind miR-200s and upregulate ZEB1 and ZEB2 which are targets of miR-200s in hepatocellular carcinoma [40]. LncRNA H19 was reported to bind miR-17–92 cluster, upregulate p21, and further suppress retinoblastoma progression [54]. LncRNA SNHG1 was reported to bind miR-145a-5p, upregulate NUAK1, and further promote the aggressiveness of NPC cells [55]. miR-122 is a well-known tumor suppressive miRNA [56–58]. Also in our previous report, we found that miR-122 suppresses NPC cell proliferation, migration, and invasion via targeting SATB1 [39]. Due to the critical tumor suppressive roles of miR-122 in NPC, we hypothesized that lncRNAs, which could competitively bind miR-122, may have oncogenic roles in NPC. In this study, we found that DRAIC has two miR-122 binding sites. The binding of DRAIC to miR-122 was verified by RNA pull-down assay, dual-luciferase reporter assays, and RIP assays. Via binding miR-122, DRAIC was found to upregulate SATB1, which was abolished by the mutation of miR-122 binding sites on DRAIC. Furthermore, the expression of SATB1 was positively correlated with that of DRAIC in NPC tissues, which supports the positive regulation of SATB1 by DRAIC. Functionally, the oncogenic roles of DRAIC in NPC cell growth, migration, and invasion were abolished by the mutation of miR-122 binding sites on DRAIC. The oncogenic roles of DRAIC were also abolished by overexpression of miR-122 or depletion of SATB1. These functional rescue experiments suggested that the roles of DRAIC are at least partially dependent on the binding of miR-122 and upregulation of SATB1. Except the DRAIC/miR-122/SATB1 regulation axis, other molecular mechanisms may also mediate the roles of DRAIC in NPC, which need further investigation.

In conclusion, these findings identified that lncRNA DRAIC was upregulated in NPC and positively correlated with clinical stages of NPC patients. DRAIC facilitated NPC cell growth, migration, and invasion via sponging miR-122 and upregulating SATB1. Our data suggested DRAIC as a potential therapeutic target for NPC.
Disclosure statement
The authors declare no conflicts of interest.

References
[1] Fan C, Tang Y, Wang J, et al. Long non-coding RNA LOC284454 promotes migration and invasion of nasopharyngeal carcinoma via modulating the Rho/Rac signaling pathway. Carcinogenesis. 2019;40:380–391.
[2] Chua MLK, Wee JTS, Hui EP, et al. Nasopharyngeal carcinoma. Lancet. 2016;387:1012–1024.
[3] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
[4] Zhang L, Huang Y, Song H, et al. Gemcitabine plus cisplatin versus fluorouracil plus cisplatin in recurrent or metastatic nasopharyngeal carcinoma: a multicentre, randomised, open-label, phase 3 trial. Lancet. 2016;388:1883–1892.
[5] Bruce JP, Yip K, Batman SV, et al. Nasopharyngeal cancer: molecular landscape. J Clin Oncol. 2015;33:3346–3355.
[6] Huang D, Bian G, Pan Y, et al. MiR-20a-5p promotes radio-resistance by targeting Rab27B in nasopharyngeal cancer cells. Cancer Cell Int. 2017;17:32.
[7] Zhang L, Madsaas KD, Zhou T, et al. Genomic analysis of nasopharyngeal carcinoma reveals TME-based subtypes. Mol Cancer Res. 2017;15:1722–1732.
[8] Mondal T, Juuvvuna PK, Kirkeby A, et al. Sense-antisense IncRNA pair encoded by Locus 6p22.3 determines neuroblastoma susceptibility via the USP36-CHD7-SOX9 regulatory axis. Cancer Cell. 2018;33:417–434 e7.
[9] Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet. 2016;17:47–62.
[10] Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet. 2014;15:7–21.
[11] Li XX, Liang XJ, Zhou LY, et al. Analysis of differential expressions of long non-coding RNAs in nasopharyngeal carcinoma using next-generation deep sequencing. J Cancer. 2018;9:1943–1950.
[12] Berger AC, Korkut A, Kanchi RS, et al. A comprehensive pan-cancer molecular study of gynecologic and breast cancers. Cancer Cell. 2018;33:690–705 e9.
[13] Li JK, Chen C, Liu JY, et al. Long noncoding RNA MRCCAT1 promotes metastasis of clear cell renal cell carcinoma via inhibiting NPM3 and activating p38-MAPK signaling. Mol Cancer. 2017;16:111.
[14] Wang Z, Yang B, Zhang M, et al. IncRNA epigenetic landscape analysis identifies EPIC1 as an oncogenic IncRNA that Interacts with MYC and promotes cell-cycle progression in cancer. Cancer Cell. 2018;33:706–720 e9.
[15] Hu WL, Jin L, Xu A, et al. GUARDIN is a p53-responsive long non-coding RNA that is essential for genomic stability. Nat Cell Biol. 2018;20:492–502.
[16] Micheliini F, Pitchiya S, Vitelli V, et al. Damage-induced IncRNAs control the DNA damage response through interaction with DDRRNAs at individual double-strand breaks. Nat Cell Biol. 2017;19:1400–1411.
[17] Zhang C, Yuan J, Hu H, et al. Long non-coding RNA CHCHD4P4 promotes epithelial-mesenchymal transition and inhibits cell proliferation in calcium oxalate-induced kidney damage. Braz J Med Biol Res. 2017;51:6536.
[18] Esposito R, Bosch N, Lanzos A, et al. Hacking the cancer genome: profiling therapeutically actionable long non-coding RNAs using CRISPR-Cas9 screening. Cancer Cell. 2019;35:545–557.
[19] Lin A, Hu Q, Li C, et al. The LINK-A IncRNA interacts with PtdIns(3,4,5)P3 to hyperactivate AKT and confer resistance to AKT inhibitors. Nat Cell Biol. 2017;19:238–251.
[20] Grelet S, Link LA, Howley B, et al. A regulated PNUTS mRNA to IncRNA splice switch mediates EMT and tumour progression. Nat Cell Biol. 2017;19:1105–1115.
[21] Yuan JH, Liu XN, Wang TT, et al. The MBNL3 splicing factor promotes hepatocellular carcinoma by increasing PXN expression through the alternative splicing of IncRNA-PXN-AS1. Nat Cell Biol. 2017;19:820–832.
[22] Huarte M. The emerging role of IncRNAs in cancer. Nat Med. 2015;21:1253–1261.
[23] Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136:629–641.
[24] Zhu XT, Yuan JH, Zhu TT, et al. Long noncoding RNA glypican 3 (GPC3) antisense transcript 1 promotes hepatocellular carcinoma progression via epigenetically activating GPC3. FEBS J. 2016;283:3739–3754.
[25] Lingadahalli S, Gadkari S, Sung YG, et al. Novel IncRNA LINCO0884 regulates prostate cancer cell migration and invasion through AR signaling. Mol Cancer Res. 2018;16:1865–1878.
[26] Yang XZ, Cheng TT, He QJ, et al. LINCO1133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/beta-catenin pathway. Mol Cancer. 2018;17:126.
[27] Kwak PB, Iwasaki S, Tomari Y. The microRNA pathway and cancer. Cancer Sci. 2010;101:2309–2315.
[28] Yan W, Wu X, Zhou W, et al. Cancer-cell-secreted exosomal miR-105 promotes tumour growth through the MYC-dependent metabolic reprogramming of stromal cells. Nat Cell Biol. 2018;20:597–609.
[29] Jiang Q, Isquith J, Zipeto MA, et al. Hyper-editing of cell-cycle regulatory and tumor suppressor RNA promotes malignant progenitor propagation. Cancer Cell. 2019;35:81–94 e7.
[30] Hydbring P, Wang Y, Fassl A, et al. Cell-cycle-targeting microRNAs as therapeutic tools against refractory cancers. Cancer Cell. 2017; 31:576–590 e8.
[31] Moro A, Driscoll TP, Boraas LC, et al. MicroRNA-dependent regulation of biomechanical genes establishes tissue stiffness homeostasis. Nat Cell Biol. 2019;21:348–358.
[32] Mu ZW, Cheng WC, Chen SF, et al. MiR-25-93 mediates hypoxia-induced immunosuppression by repressing cGAS. Nat Cell Biol. 2017;19:1286–1296.
[33] Tsaiow KM, Pearson DS, Wu Z, et al. LIN28 phosphorylation by MAPK/ERK couples signalling to the post-transcriptional control of pluripotency. Nat Cell Biol. 2017;19:60–67.
[34] Kafshdooz L, Pourfathie H, Akbarzadeh A, et al. The role of microRNAs and nanoparticles in ovarian cancer: a review. Artif Cells Nanomed Biotechnol. 2018;46:241–247.
[35] Li L, Liang Y, Kang L, et al. Transcriptional regulation of the Warburg effect in cancer by SIX1. Cancer Cell. 2018;33:368–385 e7.
[36] Yuan JH, Yang F, Chen BF, et al. The histone deacetylase 4/SP1/micromorona-200a regulatory network contributes to aberrant histone acetylation in hepatocellular carcinoma. Hepatology. 2011;54:2025–2035.
[37] Bu Y, Yoshida A, Chitnis N, et al. A PERK-miR-211 axis suppresses circadian regulators and protein synthesis to promote cancer cell survival. Nat Cell Biol. 2018;20:104–115.
[38] Javadi H, Lotfi AS, Hosseinkhani S, et al. The combinational effect of E6/E7 siRNA and anti-miR-182 on apoptosis induction in HPV16-positive cervical cells. Artif Cells Nanomed Biotechnol. 2018;46:727–736.
[39] Liu YH, Liu JL, Wang Z, et al. MiR-122-5p suppresses cell proliferation, migration and invasion by targeting SATB1 in nasopharyngeal carcinoma. Mol Cancer. 2018;20:247.
[40] Yu Y, Yang F, Wang F, et al. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci. 2019;23:622–629.
[41] Yuan JH, Yang F, Wang F, et al. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell. 2014;25:666–681.
[42] Li Y, Lv M, Song Z, et al. Long non-coding RNA NNT-AS1 affects progression of breast cancer through miR-142-3p/ZE81 axis. Biomed Pharmacother. 2018;103:939–946.
[43] Tiessen I, Abildgaard MH, Lubas M, et al. A high-throughput screen identifies the long non-coding RNA DRAC1 as a regulator of autophagy. Oncogene. 2019;38:5127.
[43] Li JH, Liu S, Zhou H, et al. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucl Acids Res. 2014;42:D92–7.

[44] Agarwal V, Bell GW, Nam JW, et al. Predicting effective microRNA target sites in mammalian miRNAs. eLife. 2015;4:e05005.

[45] Iyer MK, Niknafs YS, Malik R, et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47:199–208.

[46] Zhang X, Yang J, Bian Z, et al. Long noncoding RNA DANCR promotes nasopharyngeal carcinoma progression by interacting with STAT3, enhancing IL-6/JAK1/STAT3 signaling. Biomed Pharmacother. 2019;113:108713.

[47] Yi L, Ouyang L, Wang S, et al. Long noncoding RNA PTPRG-AS1 acts as a microRNA-194-3p sponge to regulate radiosensitivity and metastasis of nasopharyngeal carcinoma cells via PRC1. J Cell Physiol. 2019;234:19088–19102.

[48] Lian Y, Xiong F, Yang L, et al. Long noncoding RNA AFAP1-AS1 acts as a competing endogenous RNA of miR-423-5p to facilitate nasopharyngeal carcinoma metastasis through regulating the Rho/Rac pathway. J Exp Clin Cancer Res. 2018;37:253.

[49] Sakurai K, Reon BJ, Anaya J, et al. The lncRNA DRAIC/PCAT29 locus constitutes a tumor-suppressive nexus. Mol Cancer Res. 2015;13:828–838.

[50] Zhao D, Dong JT. Upregulation of long non-coding RNA DRAIC correlates with adverse features of breast cancer. ncRNA. 2018;4:39.

[51] Rajasekaran S, Nagarajha Selvan LD, Dotts K, et al. Non-coding and coding transcriptional profiles are significantly altered in pediatric retinoblastoma tumors. Front Oncol. 2019;9:221.

[52] Cesana M, Caciarelli D, Legnini I, et al. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell. 2011;147:358–369.

[53] Salmena L, Poliseno L, Tay Y, et al. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 2011;146:353–358.

[54] Zhang A, Shang W, Nie Q, et al. Long non-coding RNA H19 suppresses retinoblastoma progression via counteracting miR-17-92 cluster. J Cell Biochem. 2018;119:3497–3509.

[55] Lan X, Liu X. LncRNA SNHG1 functions as a ceRNA to antagonize the effect of miR-145a-3p on the down-regulation of NUAK1 in nasopharyngeal carcinoma cell. J Cell Mol Med. 2019;23:2351–2361.

[56] Kutay H, Bai S, Datta J, et al. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. J Cell Biochem. 2006;99:671–678.

[57] Su R, Cao S, Ma J, et al. Knockdown of SOX2OT inhibits the malignant biological behaviors of glioblastoma stem cells via up-regulating the expression of miR-194-5p and miR-122. Mol Cancer. 2017;16:171.

[58] Rao M, Zhu Y, Zhou Y, et al. MicroRNA-122 inhibits proliferation and invasion in gastric cancer by targeting CREB1. Am J Cancer Res. 2017;7:323–333.