

## ORIGINAL ARTICLE

# LncRNA DLX6-AS1 increases the expression of HIF-1 $\alpha$ and promotes the malignant phenotypes of nasopharyngeal carcinoma cells via targeting MiR-199a-5p

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## Abstract

**Objective:** To investigate the expression of long-chain noncoding growth stasis specific protein 6 antisense RNA1 (lncRNA DLX6-AS1) in nasopharyngeal carcinoma (NPC) tissues and cells, and its regulatory effect on malignant phenotypes of NPC cells.

**Methods:** The expressions of DLX6-AS1, miR-199a-5p, and HIF-1 $\alpha$  mRNA in NPC tissues and cells were detected by qRT-PCR. The proliferation, metastasis, and invasion of cells were monitored via MTT and transwell assay. The interactions between DLX6-AS1 and miR-199a-5p, miR-199a-5p and HIF-1 $\alpha$  were verified by luciferase activity assay. Western blot was performed to determine the regulatory effect of DLX6-AS1 and miR-199a-5p on HIF-1 $\alpha$  protein.

**Results:** The expression of lncRNA DLX6-AS1 was up-regulated in NPC tissues and cells. The proliferation, migration, and invasion of NPC were enhanced by overexpressed DLX6-AS1 but inhibited by DLX6-AS1 knockdown. In addition, DLX6-AS1 can be used as a kind of ceRNA to regulate miR-199a-5p and, thereby modulating the expression of HIF-1 $\alpha$ .

**Conclusion:** We found that DLX6-AS1 was a cancer-promoting lncRNA to facilitate the progression of NPC, and its underlying mechanism was suppressing miR-199a-5p expression. This study can provide novel clues for the treatment of NPC.

## KEYWORDS

DLX6-AS1, HIF-1 $\alpha$ , Long noncoding RNA, miR-199a-5p, Nasopharyngeal carcinoma

## 1 | INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a type of head and neck cancer, which is vastly more common in certain regions of southern China and southeast Asia than elsewhere. It is estimated that there are over 60,000 newly diagnosed cases with

NPC every year in China, and such a public health issue has been brought into the center of people's concern (Tan, Tang, & Tang, 2015). A slew of patients with primary NPC remain gripped by the relapse and distant metastasis, despite of the fact that they are sensitive to radiotherapy and chemotherapy (Cheng et al., 2000). Notwithstanding the tremendous

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achievements in radiotherapy and chemotherapy, these approaches only provide very limited survival advantages due to the relapse and distant metastasis (Liu et al., 2017). Correspondingly, it's imperative to probe into the biological mechanism of NPC.

Long noncoding RNA (lncRNA) is a class of non-coding RNA (ncRNA) that is more than 200 nucleotides without protein-coding ability. lncRNA, failing to translate proteins, wields enormous influence in biological processes, such as the proliferation, apoptosis, metastasis, tumorigenesis, and progression (Batista & Chang, 2013; Dhamija & Diederichs, 2016; Ponting, Oliver, & Reik, 2009). A growing body of evidences demonstrate that lncRNAs can modulate tumor progression (Adams, Parsons, Walker, Zhang, & Slack, 2017; Smolle, Bauernhofer, Pummer, Calin, & Pichler, 2017; Wapinski & Chang, 2011). For instance, lncRNA 00,858, as a competitive endogenous RNA of miR-422a, potentiates the cell growth of nonsmall cell lung cancer (Zhu, Wang, Wang, & Zhao, 2017). The increased expression of lncRNA ZFAS1 is related to epithelial-mesenchymal transformation of gastric cancer (Zhou et al., 2016). lncRNA LOC100129148 and EWSAT1 performs a carcinogenic role in NPC (Song & Yin, 2016; Sun, Peng, Chen, Song, & Zhou, 2017). It has been well documented that high expression of long-chain non-coding growth stasis specific protein 6 anti-sense RNA1 (lncRNA DLX6-AS1) (HGNC#37151) is associated with tissue differentiation and TNM stage of lung cancer (Li et al., 2015). DLX6-AS1 can also accelerate tumor proliferation and metastasis in osteosarcoma (Zhang, Meng, Mei, Zhao, & Chen, 2019). The expression of DLX6-AS1 is up-regulated and is correlated with poor prognosis in patients with hepatocellular carcinoma (Zhang, He, Jin, Gang, & Jin, 2017). Nevertheless, the role of DLX6-AS1 in NPC remains far from being thoroughly elucidated.

miRNA is a class of highly conserved noncoding RNA molecules of 21 ~ 25nt length, which are involved in the regulation of gene transcription and expression by binding directly to the target mRNA (Voinnet, 2009). The suppressive influences that miR-199a-5p and miR-199b-5p impose on the progression of hepatocellular carcinoma by inhibiting *ROCK1* have been revealed in previous articles (Zhan et al., 2017). miR-199a-5p restrains the proliferation but enhances the apoptosis of hemangioma cells (Wang et al., 2018). Additionally, miR-199b-5p facilitates the growth and metastasis of cervical cancer by down-regulating *KLK10* (Xu, Duan, Wang, & Yin, 2018).

There is accumulating evidence that lncRNA can interact directly with miRNA and regulate their expression and activity (Bayoumi et al., 2016; Cao, Jiang, Tang, & Liang, 2017). This study indicated that, in NPC tissues, the expression of DLX6-AS1 was up-regulated compared with paired

adjacent normal nasopharyngeal epithelial tissues, and contributed to inhibiting the expression of miR-199a-5p. Moreover, both knockdown of DLX6-AS1 and overexpressed miR-199a-5p repressed the proliferation, migration and invasion of NPC cells in vitro. Luciferase reporter gene assay uncovered that *HIF-1 $\alpha$*  was a direct target of miR-199a-5p in NPC cells. These results and findings implied a underlying DLX6-AS1/miR-199a/b-5p/*HIF-1 $\alpha$*  pathway and provided new insights into the tumorigenesis and progression of NPC.

## 2 | MATERIALS AND METHODS

### 2.1 | General materials

72 patients with NPC who underwent biopsy in our hospital from 2012 to 2017 were enrolled in this study. Of the patients, were 44 male and 28 female, the age of which ranged from 21 ~ 68 years old. The NPC tissue samples and non-NPC tissues were taken and confirmed by pathological examination after operation. Neither the radiotherapy and chemotherapy nor targeted drugs were given to any patients. Prior patient consents were obtained. The collected cancer tissues and normal tissues were frozen at  $-80^{\circ}\text{C}$ .

### 2.2 | Cell culture

Nasopharyngeal epithelial cells NP69, NPC cell lines S18, S26, CNE-1, CNE-2, HONE-1, and 5-8F were purchased from Shanghai Enzyme Research Biotechnology Co, Ltd. The cells were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco) and 100 U/ml penicillin/streptomycin (Life Technologies, USA) at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ .

### 2.3 | RT-qPCR

Total RNA was extracted from cancer cells by using RNAiso Plus reagent (Takara Biotechnology Co., Ltd, Dalian). In order to detect the expression of miR-199a/b-5p, the total RNA was reversely transcribed by MMLV. cDNA taken as a template, real-time PCR was conducted using PCR kit (Qiagen). The transcripts were quantified by real-time PCR and standardized as *GAPDH* expression. With regard to the expression analysis of DLX6-AS1 and *HIF-1 $\alpha$*  mRNA, the first chain cDNA was synthesized by using cDNA synthesis kit (Takara) according to the manufacturer's instructions. cDNA expression at mRNA level was detected by Syber Green PCR Master Mix (Applied Biosystems). The comparative cycle threshold (Ct) was employed to calculate the relative expression of genes ( $2^{-\Delta\Delta\text{Ct}}$ ). The specific primer sequences of *GAPDH* were shown in Table 1.

**TABLE 1** qRT-PCR primer sequences

Name	Primer sequences
lncRNA DLX6-AS1	Forward: 5'-AGTTTCTCTCTAGATTGCCTT-3'
	Reverse: 5'-ATTGACATGTTAGTGCCCTT-3'
GAPDH	Forward: 5'-AGCCACATCGCTCAGACAC-3'
	Reverse: 5'-GCCCAATACGACCAAATCC-3'

## 2.4 | Genotyping

The GenBank reference for DLX6-AS1 is NC\_000007.14, and HIF-1 $\alpha$  is NC\_000014.9.

## 2.5 | Cell construction

In accordance with the database of National Biotechnology Information Center, DLX6-AS1 sequence lacking poly-A tail was synthesized and subcloned into pcDNA3.1 (Jikai, Shanghai, China). Using siRNA-DLX6-AS1, the expression of DLX6-AS1 was knocked down. According to the manufacturer's instructions, no-load plasmid, pcDNA-DLX6-AS1 or si-DLX6-AS1 was transfected into C666-1 and HNE1 cells cultured in the 6-well plate using Lipofectamine<sup>TM</sup> 2000 (Invitrogen). After 24 hr or 48 hr of transfection, the cells were harvested and processed for further analysis.

## 2.6 | MTT assay

The exponentially grown NPC cells were cultured in 96-well plate for 12 hr. Within 24 hr, siRNA was added to interfere with DLX6-AS1 expression, and siRNA transfection was carried out with Lipofectamine RNAiMAX reagent (Invitrogen). The medium was replaced at a specified time with a complete medium encompassing 10  $\mu$ l MTT reagent, and then the cells were incubated at 37°C for 1 hr. Vmax microspectrophotometer (Molecular Devices) was placed to measure absorbance at 570 nm.

## 2.7 | Transwell assay

The cells were inoculated in a 6-well plate equipped with chambers, and two wells were set in each group. NPC cells were suspended in the upper chamber (approximately 10<sup>5</sup> cells/well) (well diameter of 8  $\mu$ m; BD Biosciences, San Jose, CA, USA) of the serum-free medium. The lower chamber was 500  $\mu$ l high glucose DMEM medium with 10% fetal bovine serum (FBS). After 24 hr, the cells on the surface of the upper chamber were gently wiped off with cotton swabs, while the cells invading the lower chamber were fixed with methanol and then stained with crystal violet. Five visual fields (including the center and periphery of the membrane) were randomly selected under an

inverted microscope. Furthermore, the number of cells invading the lower chamber were counted. Matrix gel was coated on the bottom of the transwell chamber during the invasion assay, and the other steps were the same as the migration assay.

## 2.8 | Dual luciferase reporter gene assay

DNA oligonucleotides and pMiR-Reporter were adopted to construct luciferase reporter vectors (pMiR-DLX6-AS1-WT/pMiR-DLX6-AS1-Mut and pMiR-HIF-1  $\alpha$ -WT/pMiR-HIF-1  $\alpha$ -Mut). pMiR-DLX6-AS1-WT or pMiR-DLX6-AS1-Mut and miR-26a mimics or negative control (NC) were co-transfected into HEK293 cells. Luciferase activity was measured by Dual Luciferase Reporter Assay Kit (Promega) at 48 h post-transfection.

## 2.9 | Western blot

RIPA lysis buffer (Beyotime, China) embodying protease inhibitor cocktail (Roche) was used to prepare NPC cell lysate. The protein sample was added for SDS-PAGE and transferred to the nitrocellulose membrane. After blocked with 5% fat-free milk, the membrane was probed with the primary antibody *HIF-1 $\alpha$*  (1:1,000 dilution, Cell Signaling Technology) and the secondary antibody GAPDH (1:2,000 dilution, Santa Cruz Biotechnology). Subsequently, the membrane was incubated with horseradish peroxidase conjugated (HRP) secondary antibody (1:2,000, Santa Cruz Biotechnology) for 1 hr. Finally, an automatic imaging system (ChemiDocXRS imaging system) was employed for development and the gray value was calculated.

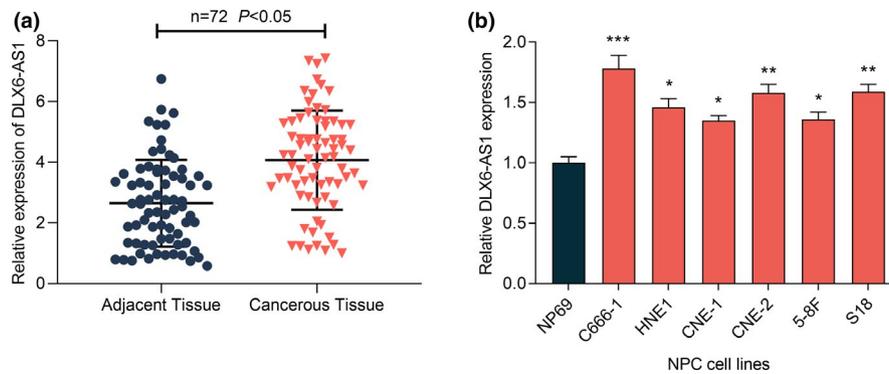
## 2.10 | Statistical analysis

All assays were performed in triplicate. The statistics data among different groups were compared by *t* test. Differences with *p* < .05 were considered to be statistically significant. All statistical analysis was carried out using SPSS17.0 software.

# 3 | RESULTS

## 3.1 | LncRNA DLX6-AS1 was highly expressed in NPC tissues and cells

First of all, we measured the expression of DLX6-AS1 in 72 cases of NPC and non-NPC tissues. As shown (Figure 1a), the expression of DLX6-AS1 was significantly up-regulated in NPC tissues compared with normal tissues. Besides, the expressions of DLX6-AS1 in all of the six selected NPC cell lines, were higher than that in nasopharyngeal epithelium cell NP69 (Figure 1b).



**FIGURE 1** lncRNA DLX6-AS1 was highly expressed in NPC tissues and cells. (a) The expressions of DLX6-AS1 in 72 pairs of NPC tissues and normal tissues were detected by qRT-PCR. (b) The expressions of DLX6-AS1 in nasopharyngeal normal epithelial cells and NPC cells were determined by qRT-PCR. \*, \*\*, \*\*\* represent  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  respectively

### 3.2 | LncRNA DLX6-AS1 could promote the proliferation, migration, and invasion of NPC cells

In order to probe into DLX6-AS1 function in NPC, we successfully established DLX6-AS1 overexpressed and knockdown cells with NPC cell lines C666-1 and HNE1 cells respectively (Figure 2a). Then we carried out MTT assay to monitor the proliferation of NPC cells at 0, 12, 24, 48, 72, and 96 hr respectively. The results implied that the proliferation of NPC cells was facilitated by the overexpression of DLX6-AS1 but restrained by the knockdown of DLX6-AS1 (Figure 2b, c). By transwell assay, we figured out that overexpressed DLX6-AS1 could expedite the migration and invasion of NPC cells (Figure 2d). By contrast, knockdown of DLX6-AS1 would hinder the migration and invasion of NPC cells (Figure 2e).

### 3.3 | miR-199a-5p was down-regulated in NPC tissues and cell lines

Multiple microRNAs were found to be dysregulated in NPC, and miR-199a-5p and miR-199b-5p were reported to be down-regulated in NPC tissues (Li, Hang, Ma, & Wu, 2016). In this study, we detected the expression levels of miR-199a-5p in NPC tissues and normal tissues by qRT-PCR. Consistent with previous reports, the expression level of miR-199a-5p in normal nasopharyngeal epithelium was lower than that in NPC tissues (Figure 3a). In addition, the expression of miR-199a-5p in normal cells was higher than that in NPC cell lines (Figure 3b).

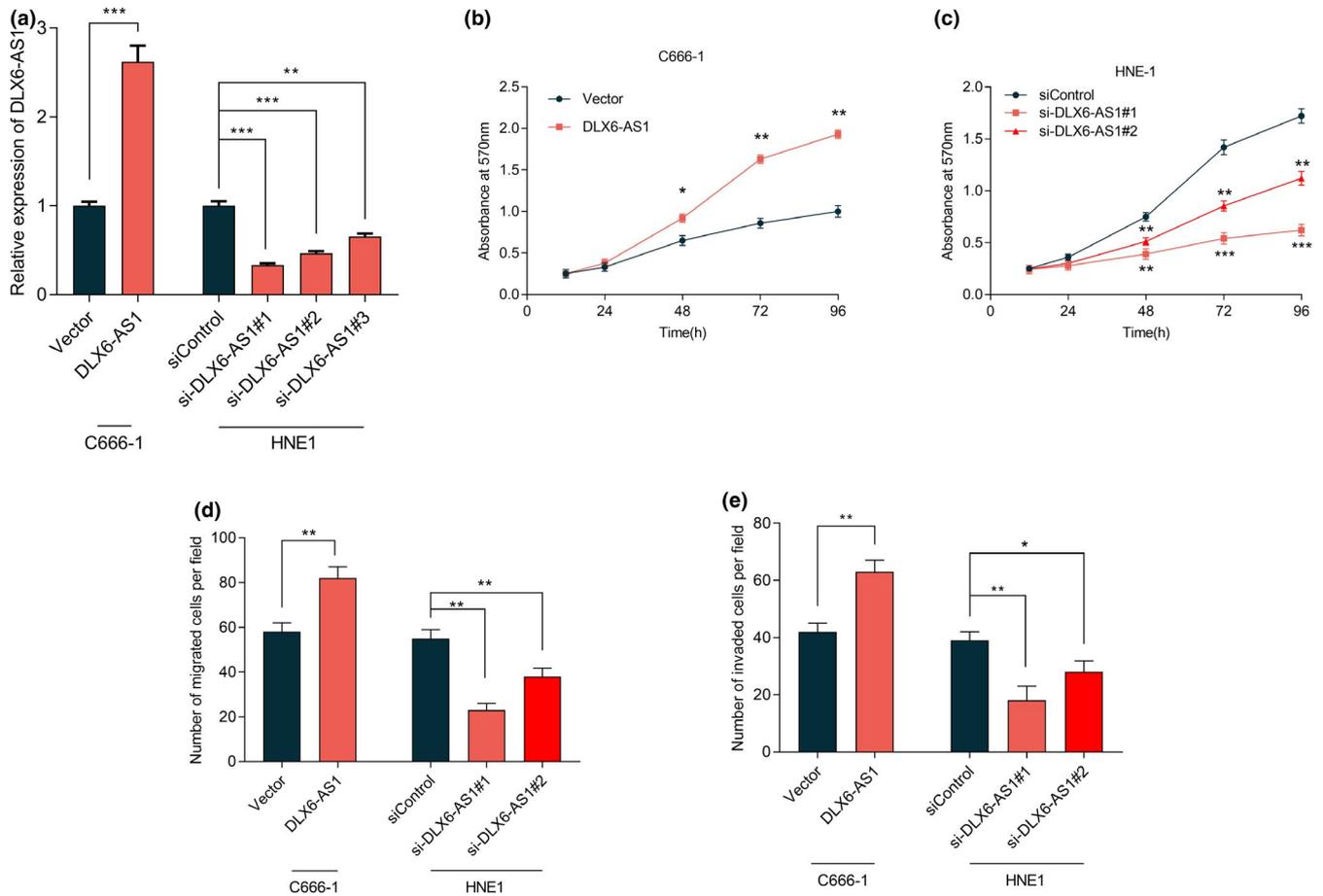
### 3.4 | miR-199a-5p inhibited the proliferation, migration and invasion of NPC cells

In order to further validate the inhibitory effect of miR-199a-5p on NPC cells, we successfully transfected

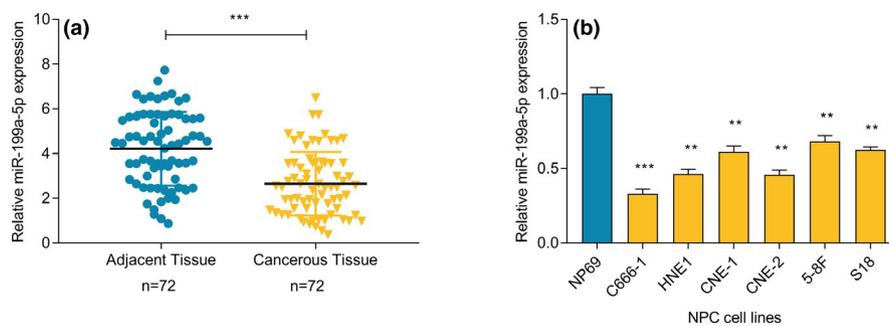
miR-199a-5p mimics and miR-199a-5p inhibitors into C666-1 cells and HNE-1 cells respectively (Figure 4a). It was confirmed by MTT assays that miR-199a-5p significantly stifled the proliferation of C666-1 cells, while its inhibitor promoted the proliferation of HNE-1 cells (Figure 4b, c). The results of transwell assays proved that miR-199a-5p could significantly inhibit the migration and invasion of NPC cells, while its inhibitor had opposite effects (Figure 4d, e).

### 3.5 | DLX6-AS1 targeted miR-199a-5p

To clarify the underlying relationship between DLX6-AS1 and miR-199a-5p, we referred to bioinformatics through StarBase (<http://www.starbase.com>). It showed that DLX6-AS1 contained a potential binding site for miR-199a-5p (Figure S1). Then we detected the expression of miR-199a-5p in NPC cell lines after DLX6-AS1 overexpression or knockdown. We noticed a decrease in miR-199a-5p expression when DLX-AS1 is overexpressed and an increase in when DLX6-AS1 is inhibited (Figure 5a). We also demonstrated that both miR-199a-5p diminished the luciferase activity of wild-type pGL3-DLX6-AS1, but imposed no notable influence on mutant-type pGL3-DLX6-AS1 (Figure 5b). Our data also exhibited that the expression level of DLX6-AS1 in NPC samples was negatively correlated with the expression level of miR-199a-5p (Figure 5c). We further explored the effect of miR-199a-5p on the carcinogenic effect of DLX6-AS1. MTT assay verified that, compared with overexpressed DLX6-AS1 group, the proliferative and metastatic abilities of NPC cells were significantly inhibited after transfection of miR-199a-5p mimics (Figure 5d, e). Taken all results together, we ensured that miR-199a-5p could bind directly to DLX6-AS1 at miRNA recognition site, and can be negatively regulated by DLX6-AS1.



**FIGURE 2** LncRNA DLX6-AS1 can promote the proliferation, migration, and invasion of NPC cells. (a) The expression of DLX6-AS1 in the constructed cells was monitored by qRT-PCR. (b) The proliferation of C666-1 cells with DLX6-AS1 overexpressed was significantly promoted, which was examined by MTT assay. (c) The proliferation of HNE-1 cells with DLX-AS1 knockdown was significantly inhibited, which was examined by MTT assay. (d) Overexpression of DLX6-AS1 promoted the migration of C666-1 cells, while DLX-AS1 knockdown inhibited the migration of HNE1 cells. (e) Overexpression of DLX6-AS1 promoted the invasion of C666-1 cells, while DLX-AS1 knockdown inhibited the invasion of HNE1 cells. \*, \*\*, \*\*\* represent  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  respectively

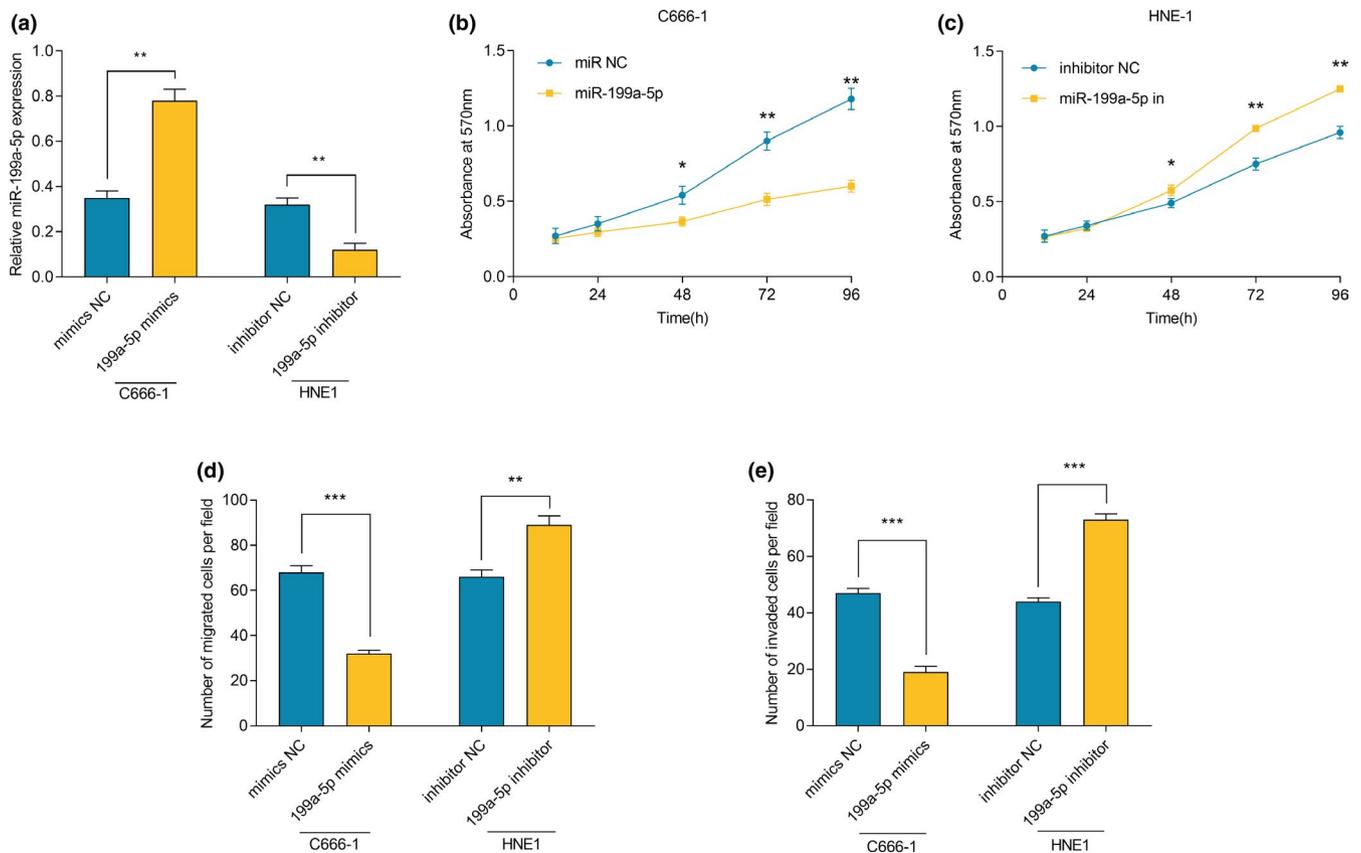


**FIGURE 3** miR-199a-5p was lowly expressed in NPC tissues and cells. (a) The expression of miR-199a-5p in 72 pairs of NPC tissues and normal tissues were detected by qRT-PCR. (b) The expression of miR-199a-5p in nasopharyngeal normal epithelial cells and NPC cells was detected by qRT-PCR. \*\*, \*\*\* represent  $P < 0.01$ ,  $P < 0.001$  respectively

### 3.6 | miR-199a-5p could inhibit the expression of *HIF-1α*, whereas lncRNA DLX6-AS1 can induce the expression of *HIF-1α*

Bioinformatics analysis also predicted that 3'-UTR of hypoxia inducible factor 1 alpha (*HIF-1α*) mRNA had 2

potential binding sites matching miR-199a-5p (Figure S1). We performed qRT-PCR assay to detect the expression of *HIF-1α* mRNA after transfection of miR-199a-5p mimics. Which was consistent with the expected, compared with control groups, the expression of *HIF-1α* mRNA in NPC cells was significantly down-regulated (Figure 6a).

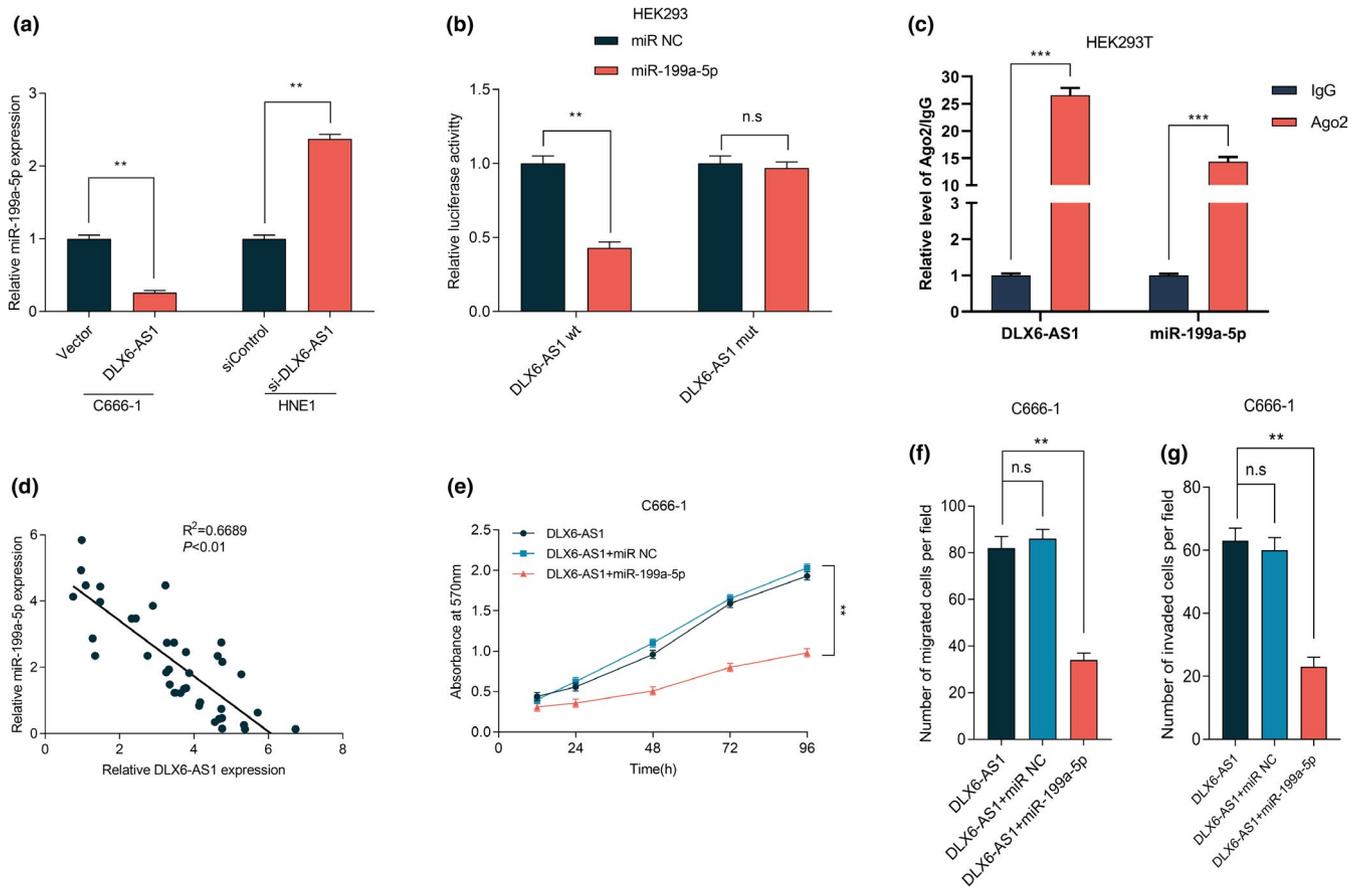


**FIGURE 4** miR-199a-5p suppressed the proliferation, migration, and invasion of NPC cells. (a) miR-199a-5p mimics and inhibitors were successfully transfected into in C666-1 cells and HNE1 cells, respectively, which was measured by qRT-PCR. (b) The proliferation of C666-1 cells transfected with miR-199a-5p inhibitors was significantly inhibited, which was examined by MTT assay. (c) The proliferation of HNE-1 cells transfected with miR-199a-5p inhibitors was significantly promoted, which was examined by MTT assay. (d) miR-199a-5p mimics inhibited the migration of C666-1 cells, while miR-199a-5p inhibitors promoted the migration of HNE1 cells. (e) miR-199a-5p mimics inhibited the invasion of C666-1 cells, while miR-199a-5p inhibitors promoted the invasion of HNE1 cells. \*, \*\*, \*\*\* represent  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  respectively

To further verify the interaction between miR-199a-5p and *HIF-1 $\alpha$* , miR-199a-5p mimics was transfected into HEK293 cells, and luciferase reporter gene assay indicated that miR-199a-5p directly targeted *HIF-1 $\alpha$*  mRNA 3'-UTR at the second binding sites (Figure 6b). Our data also exhibited that the expression level of miR-199a-5p in NPC samples was negatively correlated with the expression level of *HIF-1 $\alpha$*  (Figure 6c). Next, we detected the effects of DLX-AS1 on the expression of *HIF-1 $\alpha$* . In C666-1 cells, after DLX-AS1 was overexpressed, the expression of *HIF-1 $\alpha$*  mRNA was up-regulated. In contrast, after knockdown of DLX-AS1, *HIF-1 $\alpha$*  mRNA was down-regulated (Figure 6d). By western blot, we also discovered that the expression levels of *HIF-1 $\alpha$*  protein was modulated by DLX-AS1 (Figure 6e). Importantly, in clinical samples, the expression of *HIF-1 $\alpha$*  was negatively associated with the expression of DLX-AS1 (Figure 6f). Collectively, these data indicated that *HIF-1 $\alpha$*  was a target gene of miR-199a-5p and could be indirectly regulated by DLX-AS1.

## 4 | DISCUSSION

Recent studies revealed that lncRNAs play a vital role in the pathogenesis of cancer and provides new biological insights into tumor progression (Cheetham, Gruhl, Mattick, & Dinger, 2013; Chen, Wei, Wang, & Sun, 2017). lncRNA had been regarded as "garbage" produced in the process of transcription, which was a manifestation of the low fidelity of RNA polymeras (Ricciuti et al., 2016). The anti-tumor function and tumor-promoting function of lncRNA have been paid much attention. The dysfunction of lncRNA is closely related to the tumorigenesis, including NPC (Chen, Wang, Zhang, & Chen, 2014). The up-regulated expression of UCA1 in pancreatic cancer promotes cell migration and invasion through Hippo signaling pathway (Zhang et al., 2018). CASC2 enhances the proliferation and metastasis of bladder cancer cells by activating wnt/ $\beta$ -catenin signaling pathway (Pei et al., 2017). By mediating miR-124/NF- $\kappa$ B, NEAT1 facilitates NPC progression (Cheng & Guo, 2017). However, the function of lncRNAs on NPC remains largely unknown. To understand the precise molecular mechanisms of lncRNA



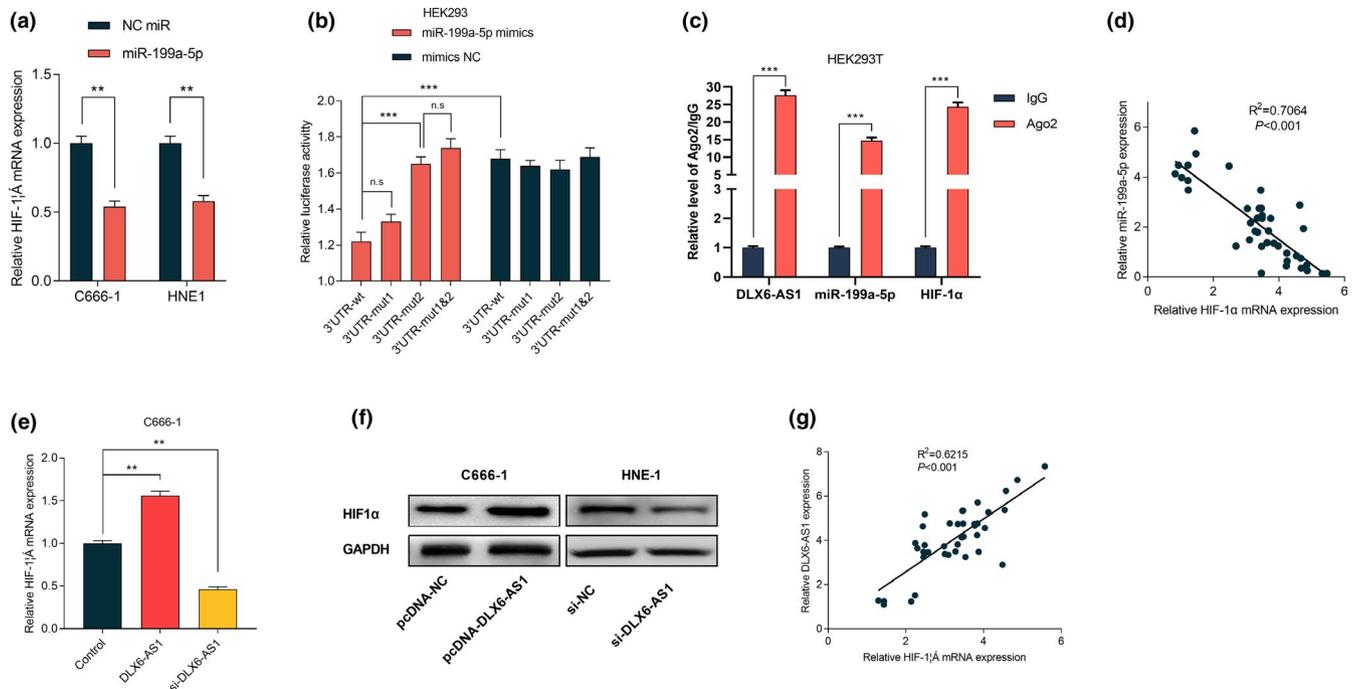
**FIGURE 5** miR-199a-5p interacted with DLX6-AS1. (a) The changes of miR-199a-5p expression after overexpression or knockdown of DLX6-AS1 were detected by qRT-PCR. (b) Dual luciferase reporter activity assay was conducted to detect the luciferase activity of wild-type and mutant DLX6-AS1 plasmids co-transfected with miR-199a-5p in HEK-293 cells. (c) The correlation between DLX6-AS1 expression and miR-199a-5p expression in clinical NPC samples was detected by qRT-PCR ( $P < 0.001$ ). (d) miR-199a-5p reversed the effect of DLX6-AS1 on proliferation in C666-1 cells. (e) miR-199a-5p reversed the effect of DLX6-AS1 on migration in C666-1 cells. (f, g) miR-199a-5p reversed the effect of DLX6-AS1 on migration and invasion in C666-1 cells. \*, \*\* represent  $P < 0.05$ ,  $P < 0.01$  respectively

function would fuel the development of lncRNA targeted therapy. LncRNA DLX6-AS1 is an antisense transcription product of DLX6 gene, and the biological function of DLX6 gene has not been elucidated (Li et al., 2015). In this study, we determined that DLX6-AS1 was up-regulated in NPC. Additionally, DLX6-AS1 knockdown significantly inhibited the proliferation, migration, and invasion of NPC cells.

miRNAs exerts great impact on the tumor progression, whose function in tumor cells has been much heralded in recent years. It has been claimed that miR-628-5p reduced the carcinogenicity of ovarian cancer cells by targeting *FGFR2* (Li et al., 2018). miR-125a produces the suppressive impact on the proliferation, migration, and invasion of cervical cancer cells via targeting *STAT3* (Fan et al., 2015). Through directly targeting *SOX9*, miR-124 represses the proliferation, migration, and invasion of lung adenocarcinoma (Wang et al., 2016). Nevertheless, the function and abnormal expression mechanism of miR-199a-5p in NPC is far from being thoroughly clarified. Mounting articles have reported that lncRNAs, as an endogenous competitive RNA, can modulate the expression

of miRNA, so lncRNA conducts a crucial role in the tumorigenesis. For instance, in gallbladder carcinoma, H19 binds to miR-342-3p to mediate *FOXXM1* expression (Wang et al., 2016). In nonsmall cell lung cancer, lncRNA UCA1 conducts a carcinogenic role via targeting miR-193a-3p (Nie et al., 2016). LncRNA DLX6-AS1 enhances the proliferation and invasion of pancreatic cancer cells by weakening the endogenous function of miR-181b in pancreatic cancer (An et al., 2018). In this study, we discovered that DLX6-AS1 facilitated the expression of *HIF-1 $\alpha$*  via targeting miR-199a-5p, thereby speeding up the proliferation and metastasis of NPC cells.

Hypoxia is one of typical microenvironments of solid tumors. Previous studies have demonstrated that hypoxia was involved in biological processes of tumor cells, such as the proliferation, apoptosis, chemotherapy resistance, angiogenesis, and metastasis (Tong, Tong, & Liu, 2018). Compared with the low expression in normal tissues, *HIF-1 $\alpha$*  expression is notably increased in hypoxic microenvironment, thus emerging as a key mediator affecting the behaviors of tumor cells (Zhang & Zhang, 2018).



**FIGURE 6** DLX6-AS1 modulated the expression of *HIF-1α* by sponging miR-199a-5p. (a) The changes of *HIF-1α* mRNA expression after transfection of miR-199a-5p mimics in C666-1 cells and HNE-1 cells were detected by qRT-PCR. (b) Dual luciferase reporter activity assay was conducted to detect the luciferase activity of wild type and mutant *HIF-1α* 3'UTR plasmids co-transfected with miR-199a-5p in HEK-293 cells. (c) The correlation between miR-199a-5p expression and *HIF-1α* expression in clinical NPC samples was detected by qRT-PCR ( $P < 0.001$ ). (d) The expression of *HIF-1α* mRNA in C666-1 cells was modulated by DLX6-AS1, which was examined by qRT-PCR. (e) The expression of *HIF-1α* protein in C666-1 cells was modulated by DLX6-AS1, which was examined by western blot. (f) The correlation between DLX6-AS1 expression and *HIF-1α* expression in clinical NPC samples was detected by qRT-PCR ( $P < 0.001$ ). (g) The correlation between DLX6-AS1 and *HIF1A* mRNA was analyzed by correlation analysis. \*\*, \*\*\* represent  $P < 0.01$ ,  $P < 0.001$  respectively

When *HIF-1α* accumulated in cells, the enhanced expression would be observed in downstream target genes such as vascular endothelial growth factor (*VEGF*) and glucose transporter 1 (*GLUT1*). Concurrently, the angiogenesis and energy metabolism were accelerated, thereby facilitating the proliferation and metastasis (Al-Anazi et al., 2018; Hamann et al., 2018). Furthermore, the abnormality of upstream regulatory molecules will also lead to the increase in *HIF-1α* expression. Our results gave the evidence that the aberrant expression of *HIF-1α* in tumor tissues may be related to the disorder of DLX6-AS1 and miR-199a/b-5p, which reveals the relationship between ncRNA and micro-environment.

In this study, we found that lncRNA DLX6-AS1 can inhibit the expression of miR-199a-5p. Some previous studies focused on that the abnormal expression of miR-199a-5p in a variety of tumor tissues can participate in the cell proliferation and motility through a variety of ways. For instance, the decreased expression of miR-199a-5p can inhibit the proliferation and invasion of ovarian cancer cells by blocking NF- $\kappa$ B signaling pathway (Liu, Yao, & Wu, 2018). miR-199a-5p can impede the proliferation and metastasis of colonic cancer cells via inhibiting *ROCK1* (Zhu et al., 2018). miR-199a-5p can also restrain the progression of thyroid carcinoma by inhibiting *SNAI1* (Ma, Jia, & Ni,

2018). Consistent with the above reports, our results proved that miR-199a-5p emerged as tumor suppressor. Its inhibitory effect can reverse the increase in cell proliferation, migration and invasion induced by DLX6-AS1 over-expression.

In conclusion, our findings provide new evidence that lncRNA DLX6-AS1 increases the expression of *HIF-1α* and promotes the malignant phenotypes of NPC cells via targeting miR-199a-5p.

## ACKNOWLEDGMENTS

None.

## CONFLICT OF INTEREST

The authors declare that they have no competing interest.

## AUTHOR CONTRIBUTION

Conceived and designed the experiments: TX; Performed the experiments: BY, LJ, CBJ, HR; Statistic analysis: QRR, DSH, HZ; Wrote the paper: LH, HYZ.

All authors read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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