

Original Research Article

HOXC10 promotes nasopharyngeal carcinoma cell proliferation and migration by regulating PI3K/AKT pathway

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Abstract

Purpose: To evaluate the role of homeobox C10 (HOXC10) in nasopharyngeal carcinoma (NPC).

Methods: Cell Counting Kit-8 (CCK-8) and colony formation assays were conducted to determine NPC cell proliferation. Cell migration and invasion were assessed using a Transwell assay, while western blot was used to investigate the mechanism of action involved in HOXC10-mediated NPC.

Results: HOXC10 levels were significantly elevated in NPC cells ($p < 0.001$). Over-expression of HOXC10 significantly increased NPC cell viability ($p < 0.05$) and proliferation. However, silencing HOXC10 reduced NPC cell proliferation. HOXC10 knockdown suppressed NPC cell migration and invasion. NPC expression of phosphorylated phosphoinositide-3-kinase (PI3K) and protein kinase B (AKT) proteins were up-regulated after HOXC10 over-expression but were down-regulated upon silencing HOXC10 ($p < 0.05$).

Conclusion: HOXC10 knockdown reduces NPC cell proliferation and metastasis by inactivating PI3K/AKT pathway, and therefore, can potentially be developed for the treatment of nasopharyngeal carcinoma.

Keywords: HOXC10, Nasopharyngeal carcinoma, Cell proliferation, Cell migration, Invasion, PI3K/AKT

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) is an epithelial malignant tumor that originates from the nasopharyngeal mucosa [1]. NPC has a high incidence and prevalence in Southeast Asia and China, which account for nearly 40% of new NPC cases worldwide [2]. Although the combination of radiotherapy and chemotherapy has significantly improved NPC treatment, patient prognosis is

poor due to the high metastasis of NPC [3]. Homeobox (HOX) genes impact embryogenesis by encoding transcriptional factors that regulate the determination of cell identity [4]. Moreover, the dysregulation of HOX genes is closely associated with human malignancies. HOX genes function either as tumor suppressors or oncogenes in diverse tumors [4]. There are four distinct clusters of HOX genes, which are HOXA, HOXB, HOXC, and HOXD. Homeobox C10

(HOXC10) belongs to the HOXC cluster and functions as an oncogene in various tumors [5]. For example, HOXC10 is up-regulated in colorectal cancer [6], and high HOXC10 expression is correlated with a poor prognosis for basal-like breast cancer [7]. HOXC10 over-expression led to the metastasis and tumorigenesis of hepatocellular carcinoma [8] and silencing HOXC10 repressed gastric cancer tumor cell proliferation and metastasis [9]. Moreover, HOXC10 also conferred resistance to chemoradiotherapy in breast cancer [10] and esophageal squamous cell carcinoma cells [11]. However, the role and mechanism of action of HOXC10 in NPC remain unclear.

The phosphoinositide-3-kinase (PI3K)/protein kinase B (AKT) pathway is constitutively activated in NPC [12], and blocking the PI3K/AKT pathway suppresses NPC metastasis [13]. HOXC10 binds to the promoter region of erb-b2 receptor tyrosine kinase 3, which promotes PI3K phosphorylation, activating the PI3K/AKT pathway [11]. Therefore, regulating the PI3K/AKT pathway may allow HOXC10 to regulate NPC tumorigenesis.

This study aimed to investigate the effect of HOXC10 on NPC cell proliferation, invasion, and migration, as well as assess the mechanism of action involved, which may ultimately reveal a potential target for NPC treatment.

EXPERIMENTAL

Cell culture and transfection

The NPC cell lines C666-1, SUNE-1, 5-8F, and 6-10B, and human nasopharyngeal epithelial cell line NP69 were grown in Roswell Park Memorial Institute (RPMI) medium 1640 (GibCo BRL, Grand Island, NY, USA) containing 10 % fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) in a 37 °C incubator. Full-length HOXC10 was subcloned into the pcDNA-3.1 vector (Invitrogen, Carlsbad, CA, USA) to generate pcDNA-HOXC10. The siRNA targeting HOXC10 (si-HOXC10) was synthesized by Genepharma (Suzhou, China). C666-1 and 5-8F cells were transfected with siRNAs (si-HOXC10 and si-NC) or pcDNAs (pcDNA-HOXC10 and pcDNA) using Lipofectamine 2000 (Invitrogen) for 24 hours.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Cells were lysed in TRIzol (Invitrogen) in order to isolate total RNA. The isolated total RNA was then reverse transcribed into cDNA using the

Multiscribe™ reverse transcription kit (Applied Biosystems, CA, USA). The mRNA expression of HOXC10 was assessed using the PreTaq II kit (Takara, Dalian, Liaoning, China) and normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control. Primer sequences are shown in Table 1.

Table 1: Primer sequences for qRT-PCR

Gene	Forward	Reverse
HOXC10	5'-	5'-
	ATCGAATTCATG	ATCCTCGAGTCAG
	ACATGCCCTCG	GTGAAATTTAAAT
	CAATGT-3'	GG-3'
GAPDH	5'-	5'-
	GGCATGGACTG	TGCACCCCAACTG
	TGGTCATGAG-3'	CTTAGC-3'

Cell viability and proliferation assays

To measure cell viability, C666-1 and 5-8F (1 × 10⁶) were seeded into a 96-well plate and transfected as indicated for 24 hours. Cells were then grown for an additional 24, 48, 72, or 96 hours. Cell counting kit 8 (CCK8) solution (Beyotime, Beijing, China) was then added to each well and incubated for 2 hours. Absorbance at 490 nm was measured using a microplate reader (Thermo Fisher Scientific). To determine cell proliferation, C666-1 and 5-8F cells were seeded in a 6-well plate and were subsequently transfected as indicated. Cells were grown in the 37 °C incubator for ten days. Cell colonies were fixed in methanol and stained with 0.1 % crystal violet (Sigma-Aldrich, St. Louis, MO, USA). The colonies were photographed under a light microscope (Olympus, Tokyo, Japan).

Transwell assay

To assess cell migration, C666-1 and 5-8F cells in serum-free medium were plated in upper transwell insert chambers (Corning Incorporated, Corning, NY, USA). Culture medium containing 15 % fetal bovine serum was plated in the lower chamber. Invasive cells in the lower chamber were stained with crystal violet and observed with a microscope (Olympus) 24 hours after plating. To determine cell invasion, C666-1 and 5-8F cells in serum-free medium were also plated in the Matrigel-coated upper chambers, and invasive cells were stained and observed using a microscope (Olympus) 24 hours post-plating.

Western blot assay

C666-1 and 5-8F cells were lysed in RIPA buffer (Beyotime). Lysates were centrifugated at 12000 × g for 60 minutes, and the supernatant was

collected. Supernatant protein concentration was determined using a BCA Protein Assay Kit (Beyotime). Protein samples of the supernatant were separated on a 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. Proteins were transferred onto nitrocellulose membranes and blocked with 5 % bovine serum albumin. The membranes were then probed at 4 °C with the following antibodies: anti-GAPDH (1:2000), anti-p-PI3K and anti-PI3K (1:3000), anti-p-AKT and anti-AKT (1:4000). The membranes were then washed using PBS-T and incubated with a horseradish peroxidase-conjugated secondary antibody (1:5000). Immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich) and normalized to GAPDH as the internal control. All antibodies were acquired from Abcam (Cambridge, MA, USA).

Statistical analysis

All data with at least three replicates are expressed as mean \pm standard error of the mean (SEM). Data were analyzed using either a Student's t-test or a one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software 11.5 (IBM, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

HOXC10 is elevated in NPC

Compared to the human nasopharyngeal epithelial cell line NP69, *HOXC10* mRNA expression was significantly up-regulated in NPC cell lines C666-1, SUNE-1, 5-8F, and 6-10B (Figure 1 A). Furthermore, *HOXC10* protein expression was also increased in NPC cells compared to NP69 (Figure 1 B), suggesting a possible relationship between *HOXC10* and NPC.

HOXC10 contributes to NPC cell proliferation

C666-1 and 5-8F cell viability increased after transfection with pcDNA-HOXC10, a construct that overexpresses *HOXC10* (Figure 2 A). However, knockdown of *HOXC10* after transfection with si-HOXC10 decreased C666-1 and 5-8F cell viability (Figure 2A). Moreover, the number of C666-1 and 5-8F colonies was enhanced by *HOXC10* over-expression but was reduced by *HOXC10* knockdown (Figure 2 B). These findings show that *HOXC10* promotes NPC cell proliferation.

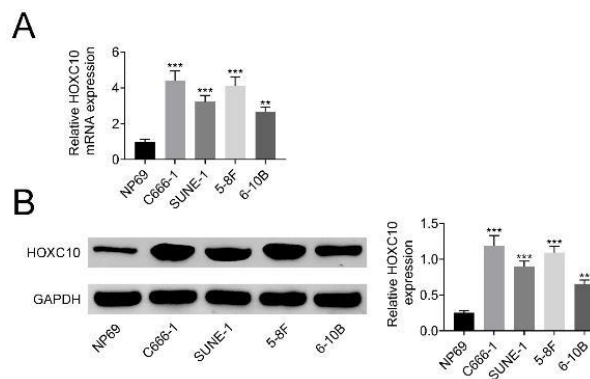


Figure 1: *HOXC10* expression is elevated in NPC cells. (A) The mRNA expression of *HOXC10* was significantly up-regulated in NPC cell lines C666-1, SUNE-1, 5-8F, and 6-10B compared to the human nasopharyngeal epithelial cell line NP69. (B) *HOXC10* protein expression was significantly up-regulated in NPC cells compared to NP69 cells. ** $P < 0.01$, *** $p < 0.001$

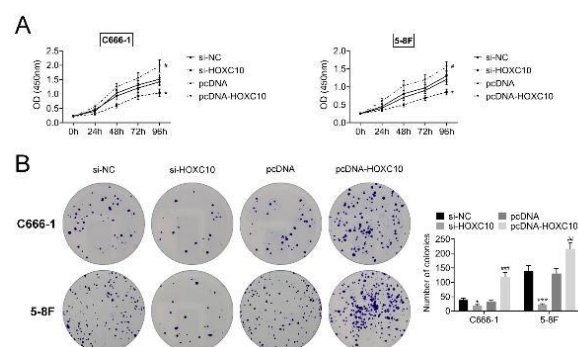


Figure 2: *HOXC10* contributes to NPC cell proliferation. (A) C666-1 and 5-8F cell viability increased after pcDNA-HOXC10 transfection and decreased after si-HOXC10 transfection. (B) C666-1 and 5-8F cell proliferation increased after pcDNA-HOXC10 transfection and decreased after si-HOXC10 transfection. *, # $p < 0.05$. ***, ###, $p < 0.001$

HOXC10 promotes NPC cell metastasis

Invasion of C666-1 and 5-8F cells was suppressed by *HOXC10* silencing and promoted by *HOXC10* over-expression (Figure 3 A). Additionally, the number of migratory C666-1 and 5-8F cells increased after *HOXC10* over-expression and decreased after *HOXC10* knockdown (Figure 3 B), indicating the metastatic effect of *HOXC10* expression in NPC.

HOXC10 stimulates PI3K/AKT activation in NPC

Either over-expressing or silencing *HOXC10* did not affect PI3K and AKT protein expression in C666-1 and 5-8F cells (Figure 4). However, expression of p-PI3K and p-AKT decreased after *HOXC10* silencing and increased upon *HOXC10*

over-expression (Figure 4), revealing that HOXC10 activates PI3K and AKT in NPC cells.

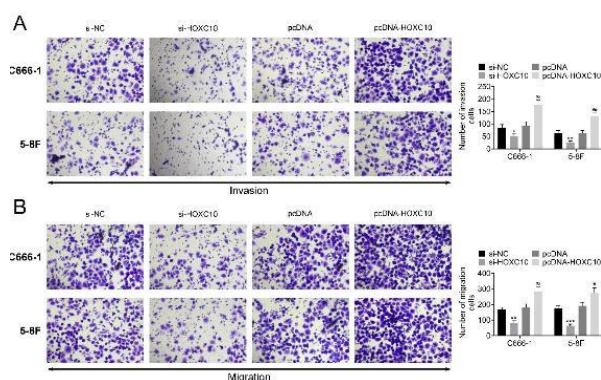


Figure 3: HOXC10 promotes NPC cell metastasis. (A) C666-1 and 5-8F cell invasion increased after transfection with pcDNA-HOXC10 and decreased after transfection with si-HOXC10. (B) Migration of C666-1 and 5-8F cells increased after transfection with pcDNA-HOXC10 and decreased after transfection with si-HOXC10. *, # $p < 0.05$. **, ## $p < 0.01$, *** $p < 0.001$

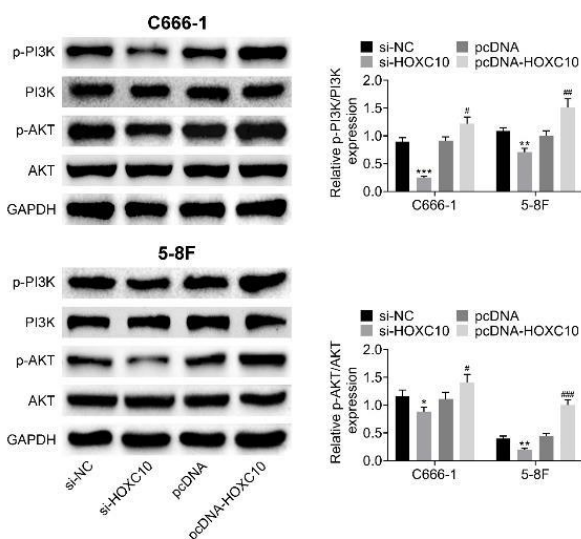


Figure 4: HOXC10 stimulates PI3K/AKT activation in NPC cells. In C666-1 and 5-8F cells, p-PI3K and p-AKT protein expression increased upon HOXC10 over-expression and decreased upon HOXC10 knockdown. *, # $P < 0.05$. **, ## $p < 0.01$. ***, ### $p < 0.001$

DISCUSSION

The dysregulation of HOX genes contributes to carcinogenesis in distinct organs, and HOX genes are regarded as a potential target for cancer prevention [4]. In NPC, HOXC6 is associated with poor patient survival [14], and HOXC8 inhibits tumor growth by regulating the glycolytic pathway [15]. Aligning with the oncogenic role of HOXC10 in various tumors [5], our present study found that HOXC10 also promotes NPC cell proliferation and metastasis.

We found that HOXC10 expression was elevated in NPC cells. High HOXC10 expression is positively correlated with poor patient prognosis and poor tumor pathological staging [5]. Therefore, HOXC10 may also function as a diagnostic or prognostic biomarker of NPC. However, the relationship between HOXC10 and the clinical indicators of NPC patients requires further investigation.

HOXC10 over-expression enhances tumor proliferation, recrudescence, invasiveness, and drug resistance [5]. Our present study also shows that NPC cell proliferation, invasion, and migration were promoted during high HOXC10 expression but were suppressed by low HOXC10 expression. Dysregulation of HOXC10 contributes to tumorigenesis through regulating cell angiogenesis and epithelial-to-mesenchymal transition [5]. Further investigation is required to understand the effects of HOXC10 on the NPC cell cycle, apoptosis, and epithelial-to-mesenchymal transition.

Aberrant activation of PI3K/AKT signaling contributes to NPC tumorigenesis and carcinogenic transformation through the regulation of autophagy, cell metastasis, apoptosis, and proliferation [16]. HOXC10 elevation enhances PI3K phosphorylation, up-regulates the genes involved in the PI3K/AKT pathway, and accelerates cancer progression [11]. Furthermore, HOXC10 knockdown reduces PI3K phosphorylation and inhibits the metastasis of lung adenocarcinoma [17].

The results of this study have demonstrated that PI3K and AKT phosphorylation were down-regulated by silencing HOXC10 and up-regulated during HOXC10 over-expression. Thus, HOXC10 promotes the aberrant activation of PI3K/AKT signaling, which intensifies tumor progression.

CONCLUSION

HOXC10 is elevated in NPC, but over-expression of HOXC10 enhances NPC cell proliferation, invasion, and migration. Moreover, HOXC10 knockdown represses NPC cell proliferation and metastasis by inactivating PI3K/AKT signaling. However, the *in vivo* effects of HOXC10 on NPC tumor growth remain to be examined.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Liping Wu and Guohong Qian designed and performed the experiments. Yuqing Zheng analyzed and interpreted the data. Huizhen Zheng prepared the manuscript with contributions from all co-authors.

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