Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

HOTAIR Promotes Cisplatin Resistance of Nasopharyngeal Carcinoma Cells by Regulating Cell Proliferation, Invasion, and Apoptosis via miR-106a-5p/SOX4 Axis

Wei Cao
The Second Hospital of Anhui Medical University
Yi Sun
The Second Hospital of Anhui Medical University
Long Liu
The Second Hospital of Anhui Medical University
Junwei Yu
The Second Hospital of Anhui Medical University
Jiabiao Ji
The Second Hospital of Anhui Medical University
Yatang Wang
The Second Hospital of Anhui Medical University
Jianming Yang (yangjianming2021@163.com)
the second hospital of anhui medical university https://orcid.org/0000-0001-8221-2469

Primary research

Keywords: LncRNA HOTAIR, miR-106a-5p/SOX4 axis, cisplatin resistance, nasopharyngeal carcinoma

Posted Date: August 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-64031/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background: Nasopharyngeal carcinoma (NPC) is a highly malignant neoplasm originating from nasopharyngeal mucosa, and the emergence of multi-drug resistance poses a huge challenge for clinical treatment of NPC. LncRNA HOTAIR (HOX antisense intergenic RNA) has been reported to be associated with many malignancies, including NPC. However, the underlying mechanisms of HOTAIR involved in drug resistance in NPC are obscure.

Methods: Quantitative polymerase chain reaction (qPCR) was employed to determine the HOTAIR, miR-106a-5p and SOX4 expression in NPC tissues and cells. The target relationship between HOTAIR and miR-106a-5p or miR-106a-5p and SOX4 was determined using dual-luciferase reporter assay. Cell proliferation, apoptosis, migration and invasion were explored using Cell counting kit-8 (CCK-8), flow cytometer and Transwell assays. The protein levels were confirmed using western blot.

Results: Our study showed that HOTAIR was upregulated in cisplatin (DDP)-resistant NPC tissues and cells. HOTAIR knockdown decreased the DDP resistance, drug resistance related gene expression, cell proliferation and invasion, and promoted apoptosis of C666-1/DDP and CNE2/DDP cells. Mechanism researches displayed that miR-106a-5p was down-regulated in DDP-resistant NPC tissues and cells. miR-106a-5p directly bound with HOTAIR and was regulated by HOTAIR. SOX4 was inhibited by miR-106a-5p at a posttranscriptional level, and the transfection of miR-106a-5p reversed the upregulation of SOX4 caused by HOTAIR overexpression. Increase or decrease of miR-106a-5p suppressed the effect of HOTAIR upregulation or downregulation on DDP resistance, cell proliferation, invasion and apoptosis of C666-1/DDP and CNE2/DDP cells. Moreover, the transfection of SOX4 siRNA reversed the decrease of DDP resistance, cell proliferation and invasion, and rescued the increase of apoptosis induced by miR-106a-5p inhibition.

Conclusions: These data suggested that HOTAIR enhanced DDP resistance of C666-1/DDP and CNE2/DDP cells by affecting cell proliferation, invasion, and apoptosis via miR-106a-5p/SOX4 axis.

Background

Nasopharyngeal carcinoma (NPC) is a highly malignant neoplasm originating from nasopharyngeal mucosa(1). According to epidemiological data, in the past few decades, due to the extensive screening of high-risk populations, advances in imaging and radiotherapy technology, and the development of other comprehensive treatments, even in high-risk areas, the death of NPC has dropped significantly(2). However, the usual treatment for NPC patients is surgery combined with multi-drug chemotherapy, such as cisplatin (DDP)(3), or doxorubicin (Dox)(4). Although combined chemotherapy has achieved a high survival rate, unfortunately, NPC is a relatively drug resistant disease. Therefore, it is better to decipher and explore the underlying molecular mechanisms of drug resistance and to find novel therapeutic targets.

Long non-coding RNAs (lncRNAs) is a type of non-coding RNA molecule with a transcript length of more

than 200 nucleotides, and together with short-chain non-coding RNA, it forms a non-coding RNA population(5, 6). With the advancement of sequencing technology and the deepening of research, people have discovered that a large number of non-coding RNAs that were thought to have no function may actually play a very important regulatory role in cell processes(7). Recently, the role of lncRNAs has also received more and more attention. In various human cancers, lncRNA HOTAIR (HOX antisense intergenic RNA, HOTAIR) has been determined to be upregulated(8). HOTAIR has been found to be significantly upregulated in breast cancer, which is then involved in the invasiveness and metastasis(9). Moreover, HOTAIR is also been determined to regulate Wnt signaling pathway for treatment of DDP-resistant non-small cell lung cancer(10). However, the biological function of HOTAIR in DDP resistance in NPC is unclear.

MicroRNAs (miRNAs), approximately 22 nucleotides in length, could target the post-transcriptional level of the gene (11). miRNAs regulate many cellular pathways and functions including cellular metabolism, differentiation, and apoptosis, so its dysregulation lead to many human diseases(12, 13). More and more studies have shown that miRNAs play key roles in cancer chemotherapy resistance, and their potential role in determining drug sensitivity or drug resistance has been confirmed by several researches(14). As a member of the miR-17 family, miR-106a-5p is shown to be abnormally expressed in various tumors, including NPC(15), liver cancer(16) and colorectal cancer(17). Recently, overexpression of SOX4 has been found to be related to the occurrence, progression, invasion and metastasis in many various tumors, indicating that SOX4 may be a major determinant of NPC progression(18, 19).

In the present study, we found that HOTAIR and SOX4 were upregulated, while miR-106a-5p was down-regulated in DDP-resistant NPC tissues and cells. Subsequent researches demonstrated that HOTAIR enhanced DDP resistance of C666-1/DDP and CNE2/DDP cells by affecting cell proliferation, invasion, and apoptosis via miR-106a-5p/SOX4 axis. This new regulatory network may provide a promising therapeutic approach for patients with DDP-resistant NPC.

Materials And Methods

Tissue Samples

32 pairs of human primary NPC tissues and their matched normal adjacent tissues were obtained from January 2017 to July 2018 in The Second Hospital of Anhui Medical University. None of them had received any preoperative radiotherapy, chemotherapy or other medical intervention. Written informed consents from all patients were obtained from the patients prior to experiments, and this study was approved by the Ethics Committee of The Second Hospital of Anhui Medical University.

Cell Culture and Transfection

The C666-1 and CNE2 cell lines were provided by the Shanghai cell bank of the Chinese Academy of Sciences (Shanghai, China). DDP-resistant NPC cell strains (C666-1/DDP and CNE2/DDP) were established from the parental cell lines C666-1 and CNE2 by using an intermittent stepwise selection protocol over 6 months, and finally exposed to 1.2 mg/mL DDP. Cells were seeded into 1640 culture

medium containing 10% fetal bovine serum (FBS, Gibco), 100 IU/ml penicillin, and 100 IU/ml streptomycin in 5% CO_2 incubator at 37 °C and 95% humidity. The siRNAs against HOTAIR and SOX4, miR-106a-5p mimic, miR-106a-5p inhibitor and their respective controls were synthesized by Gene Pharma (Shanghai, China). Full-length cDNA of HOTAIR was inserted into the pcDNA3.1 empty vector. The cells were transfected using Lipofectamine® 3000 with HOTAIR siRNA, SOX4 siRNA, miR-106a-5p mimic, miR-106a-5p inhibitor, pcDNA3.1-HOTAIR or their respective controls.

Apoptosis assay

Apoptosis analysis was performed by using flow cytometer. Cells were collected and washed with PBS. Then they were resuspended, and 5 μL Annexin V-FITC and 5 μL PI were sequentially added. Cell apoptosis was detected by flow cytometry.

Cell Proliferation Assay

The cell concentration was adjusted to 3×10^4 / mL, and 100 μl per well was seeded in a 96-well culture plate. Each group was provided with 5 duplicate wells, which were cultured at 37°C for 48 hours. After 48 h of transfection, 96-well plates were removed, 10μl of CCK-8 solution was added to each well, and incubation was continued for 1 h. At 450 nm, the absorbance (A value) of each well was measured on a microplate reader, and the results were recorded. The experimental results were calculated as follows: cell viability (%) = 100% × (A value of each experimental group / A value of the cell control group).

Quantitative polymerase chain reaction (qPCR)

Total RNA extraction was conducted using Trizol Reagent (Shanghai Pufei Biotech Co., Ltd., Shanghai, China) to detect the relative expression of genes. The prepared cDNA was amplified using SYBR Green Master Mixture (Takara, Otsu, Japan), of which the results were calculated by LightCycler® 480 real-time PCR system (Roche, Indianapolis, Ind). The thermocycling conditions were applied as follows: DNA regeneration at 95°C for 5 min, 40 cycles at 95°C for 30 sec, followed by primer annealing at 60°C for 30 sec and primer extension at 72°C for 5 min.

Transwell Assay

Before the experiment, the cells were starved for 24 h and then collected. 100 μl of cell (6×10^5/ml) suspension was placed in the upper chamber of Transwell chamber. After incubated for 48 h, the cells adhering to the lower surface were fixed in methanol for 15 min and stained using 0.1% crystal violet for 30 min. The cells were counted under a light microscope.

Luciferase assay

The wild-type HOTAIR-3’-UTR (WT-HOTAIR-3’-UTR) or SOX4-WT (WT-SOX4-3’-UTR) and mutant HOTAIR-3’-UTR (MUT-HOTAIR-3’-UTR) or SOX4-MUT (MUT-SOX4-3’-UTR) containing the miR-106a-5p binding sites were cloned into the firefly luciferase-expressing psicheck2 vector (Promega, WI, USA) for luciferase reporter experiments and then co-transfected into cells. Luciferase assay was performed the firefly luciferase 48h post-transfection.

Western blotting

After washed with PBS, NPC tissues and cells were dissolved in commercial RIPA buffer. We got the supernatant after centrifugation at 12,000 rpm for 20 minutes. And then about equal amount of protein was loaded with 10% SDS-PAGE and transferred onto the PVDF membranes. The PVDF membranes were blocked and they were incubated overnight with primary antibodies at 4°C and then incubated for 2h at room temperature with a second anti-body temperature bound by horseradish peroxidase. Finally, the Pierce^{TM} ECL Western Blotting Substrate was added to completely cover the membrane and the collected images were analyzed by Image J.

Statistical Analysis

Values are expressed as mean±standard deviation. SPSS 20.0 was applied to analyze statistical data. Student t-test was used to indicate the difference between two groups while one-way ANOVA was performed for the difference between three or multiple groups. Each assay was repeated at least three times and $P<0.05$ was accepted as statistically significant.

Results

HOTAIR was upregulated in DDP-resistant NPC tissues and cells

Firstly, qPCR was performed to detect the expression of HOTAIR in NPC tissues and cells. We found that HOTAIR was upregulated in DDP-sensitive and DDP-resistant NPC tissues compared to that in paracancerous tissues (Fig. 1A). Also, the data showed higher HOTAIR expression in C666-1/DDP and CNE2/DDP cells in comparison to that in C666-1 and CNE2 cells (Fig. 1B).

Down-regulation of HOTAIR decreased the resistance of C666-1/DDP and CNE2/DDP cells to DDP

To explore the role of HOTAIR played on NPC chemoresistance, the siRNA specifically against HOTAIR was transfected into C666-1/DDP and CNE2/DDP cells. In the first, we determined the transfection efficiency of si-HOTAIR (Fig.2A). Then the IC50 values of DDP in C666-1/DDP (or CNE2/DDP) cells were observably increased compared with C666-1 (or CNE2) cells, but significantly decreased after the interference of HOTAIR. In addition, we confirmed that HOTAIR knockdown in C666-1/DDP and CNE2/DDP cells effectively decreased the protein levels of MDR1, MRP5, LRP1 and ABCB1, which were multi-drug resistance related genes (Fig. 1D and E).

Interference with HOTAIR inhibited cell proliferation, invasion and promoted apoptosis of C666-1/DDP and CNE2/DDP cells

Based on the above results, the effect of HOTAIR in C666-1/DDP and CNE2/DDP cells was further investigated. The results showed that the cell proliferative and invasive ability were prominently suppressed, but the apoptosis was increased in C666-1/DDP and CNE2/DDP cells by reason of the decrease of HOTAIR (Fig. 3A-E).

miR-106a-5p was down-regulated in DDP-resistant NPC tissues and cells and regulated by HOTAIR

The results displayed that miR-106a-5p was dramatically down-regulated in DDP-sensitive and DDP-resistant NPC tissues, C666-1/DDP and CNE2/DDP cells in contrast to that in their matched controls (Fig.

4A and B). Next, StarBase v2.0 online database indicated that miR-106a-5p contains the potential binding site for HOTAIR (Fig.4C). And luciferase reporter gene assay indicated that luciferase activity of HOTAIR-WT in cells transfected with miR-106a-5p mimic resulted in the decline, but not of the HOTAIR-MUT reporter (Fig.3D). Furthermore, as shown in Fig. 4E, the inhibition of HOTAIR significantly promoted the expression of miR-106a-5p in C666-1/DDP and CNE2/DDP cells.

HOTAIR regulated the DDP resistance, cell proliferation, invasion and apoptosis of C666-1/DDP and CNE2/DDP cells by binding miR-106a-5p

HOTAIR overexpression vector was transfected into C666-1/DDP and CNE2/DDP cells alone or together with miR-106a-5p mimic. We demonstrated that abnormally upregulation of miR-106a-5p reversed the increase of IC50 values (Fig. 5A and B). The cell proliferation was also decreased when overexpression the miR-106-5p (Fig. 4C and D). Moreover, compared with the HOTAIR-overexpressed group, the transfection of miR-106a-5p mimic promoted apoptosis (Fig. 4E). The invasion and migration induced by HOTAIR overexpression in C666-1/DDP and CNE2/DDP cells were inhibited by overexpression of miR-106-5p (Fig. 5F and G).

SOX4 was a target of miR-106a-5p

We observed that SOX4 was upregulated in DDP-sensitive and DDP-resistant NPC tissues, C666-1/DDP and CNE2/DDP cells compared to that in their respective controls (Fig. 6A and B). TargetScan Human 7.2 indicated that miR-106a-5p contains the potential binding site for SOX4 (Fig.6C). Following dual-luciferase reporter gene assay displayed that miR-106a-5p overexpression led to a significantly decline in the luciferase activities of SOX4-WT and SOX4-MUT (Fig.6D). In addition, overexpression of HOTAIR promoted SOX4 expression, whereas transfection of miR-106a-5p mimic abolished this effect in C666-1/DDP and CNE2/DDP cells (Fig. 6E and F).

HOTAIR regulated the DDP resistance, cell proliferation, invasion and apoptosis of C666-1/DDP and CNE2/DDP cells via miR-106a-5p/SOX4 axis

Next, we investigated whether HOTAIR exerted its effects in C666-1/DDP and CNE2/DDP cells via regulating miR-106a -5p/SOX4 axis. The results showed that the transfection of miR-106a -5p inhibitor suppressed the decline of IC50 values (Fig. 7A and B), In contrast, the inhibition of miR-106a-5p reversed the increase of apoptosis caused by HOTAIR interference, and the inhibition of SOX4 reversed the reduction of apoptosis caused by miR-106a-5p interference (Fig. 7C). The decrease of cell proliferation, invasion and migration caused by HOTAIR interference and the transfection of SOX4 siRNA were impeded by miR-106a -5p inhibitor in C666-1/DDP and CNE2/DDP cells (Fig. 7D and E).

Discussion

DDP has been widely used in the treatment of various solid tumors. It is a metal-containing drug, which makes it non-cross-resistance with other drugs in certain key pathways(20). Therefore, the mere elaboration of the mechanism of NPC resistant to DDP alone has important therapeutic value. In this study, we aimed to explore the mechanism and function of HOTAIR in the DDP resistance in NPC. Our

results suggested that HOTAIR knockdown reduced the DDP resistance of C666-1/DDP and CNE2/DDP cells.

LncRNAs (more than 200bp in length) are most highly conserved in sequence and play a role in multiple functions of cells. Abnormal expression of related lncRNA has been found in many diseases, including many types of cancer(21). This phenomenon indicates that abnormal expression of lncRNAs may play an important role in carcinogenesis and cancer progression. HOTAIR has been reported to decrease the p21WAF1/CIP1 expression to regulate the DDP resistance in human lung adenocarcinoma cells(22). Moreover, HOTAIR also targeted miR-126 to activate the PI3K/AKT/MRP1 signaling pathway to promote DDP resistance in gastric cancer(23). Our data showed that HOTAIR interference decreased the DDP resistance and drug resistance related gene expression of C666-1/DDP and CNE2/DDP cells, which was consistent with previous studies. HOTAIR is up-regulated in clinical NPC tissue specimens, suggesting that HOTAIR is a potential NPC molecular marker. This study revealed that HOTAIR was upregulated both in DDP-resistant NPC tissues and cells. HOTAIR silencing inhibited the proliferation, adhesion, migration and invasion of NPC cells, which may play a role in tumor suppression in NPC cells. Moreover, in the present study, we demonstrated that the effect of HOTAIR on NPC-resistant cell proliferation and invasion in the NPC cells, and HOTAIR might act by targeting the miR-106a-5p/SOX4 axis. In brief, HOTAIR was involved in the DDP resistance in NPC cells.

miRNAs are reported to be novel DDP sensitivity modulators. Recent studies have shown that decreased miR-26b expression could repress JAG1 to induce DDP resistance in NPC(24). As previous research has shown, miR-106a-5p could target FASTK to promote apoptosis associated with the inhibition of proliferation and migration in astrocytoma cells(25). And miR-106a-5p suppresses the proliferation, migration, and invasion of osteosarcoma cells by targeting HMGA2(26). In our study, miR-106a-5p was determined to be down-regulated in DDP-resistant NPC tissues and cells, and its overexpression or inhibition reversed the effect of HOTAIR upregulation or downregulated on DDP resistance, cell proliferation, invasion and apoptosis of C666-1/DDP and CNE2/DDP cells. What's more, SOX4 was targeted by miR-106a-5p, and miR-106a-5p might play its role by targeting SOX4. SOX4 is upregulated in multiple cancer tissues. In this study, we testified in this article that SOX4 was regulated by miR-106a-5p and HOTAIR, and SOX4 knockdown reversed the effect of miR-106a-5p inhibition on DDP sensitivity, proliferation, invasion and apoptosis of C666-1/DDP and CNE2/DDP cells, and revealing that SOX4 was closely related to the resistance of cells to DDP in NPC.

In conclusion, lncRNA HOTAIR and SOX4 were markedly up-regulated, while miR-106a-5p was notably down-regulated in DDP-resistance NPC tissues, C666-1/DDP and CNE2/DDP cells. Mechanism studies confirmed that HOTAIR was involved in the formation of DDP resistance in NPC via the miR-106a-5p/SOX4 axis. This may offer a promising therapeutic strategy to DDP-resistant NPC.

Abbreviations

NPC: Nasopharyngeal carcinoma;

HOTAIR: HOX antisense intergenic RNA;

qPCR: Quantitative polymerase chain reaction;

CCK-8: Cell counting kit-8;

DDP: cisplatin;

Dox: doxorubicin;

lncRNAs: Long non-coding RNAs;

miRNAs: MicroRNAs

Declarations

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Second Hospital of Anhui Medical University. Written informed consent was obtained from all enrolled subjects.

Consent to publish

Not applicable.

Availability of data and materials

The data used to support the findings of this study are included in the article.

Competing Interest

There are no conflicts of interest to declare.

Funding

Not applicable.

Authors' Contributions

W.T., Y.S, L.L., Y.T.W., and J.M.Y. wrote the manuscript and operated the experiments; W.T., J.W.Y., and J.B.J. operated molecular experiments; W.T. and Y.S. analysed the data and designed the experiments and edited the manuscript.

Acknowledgement

Not applicable.

References

1. Lee AWM, Ng WT, Chan JYW, et al.: Management of locally recurrent nasopharyngeal carcinoma. Cancer treatment reviews 79: 101890, 2019.
2. Sun XS, Li XY, Chen QY, Tang LQ and Mai HQ: Future of Radiotherapy in Nasopharyngeal Carcinoma. The British journal of radiology 92: 20190209, 2019.
3. Zhang Y, Chen L, Hu GQ, et al.: Gemcitabine and Cisplatin Induction Chemotherapy in Nasopharyngeal Carcinoma. 381: 1124-1135, 2019.
4. Altundag K, Aksoy S, Gullu I, et al.: Salvage ifosfamide-doxorubicin chemotherapy in patients with recurrent nasopharyngeal carcinoma pretreated with Cisplatin-based chemotherapy. Medical oncology (Northwood, London, England) 21: 211-215, 2004.
5. Kondo Y, Shinjo K and Katsushima K: Long non-coding RNAs as an epigenetic regulator in human cancers. Cancer science 108: 1927-1933, 2017.
6. Beermann J, Piccoli MT, Viereck J and Thum T: Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. Physiological reviews 96: 1297-1325, 2016.
7. Mercer TR, Dinger ME and Mattick JS: Long non-coding RNAs: insights into functions. Nat Rev Genet 10: 155-159, 2009.
8. Qu X, Alsager S, Zhuo Y and Shan B: HOX transcript antisense RNA (HOTAIR) in cancer. Cancer Lett 454: 90-97, 2019.
9. Zhao W, Geng D, Li S, Chen Z and Sun M: LncRNA HOTAIR influences cell growth, migration, invasion, and apoptosis via the miR-20a-5p/HMGA2 axis in breast cancer. 7: 842-855, 2018.
10. Guo F, Cao Z, Guo H and Li S: The action mechanism of lncRNA-HOTAIR on the drug resistance of non-small cell lung cancer by regulating Wnt signaling pathway. Experimental and therapeutic medicine 15: 4885-4889, 2018.
11. Poel D, Boyd LNC and Beekhof R: Proteomic Analysis of miR-195 and miR-497 Replacement Reveals Potential Candidates that Increase Sensitivity to Oxaliplatin in MSI/P53wt Colorectal Cancer Cells. 8, 2019.
12. Xu T, Yu W, Li Q, et al.: MicroRNA-524 inhibits the progress of glioma via the direct targeting of NCF2. Am J Transl Res 11: 1605-1615, 2019.
13. Chang H, Li J, Qu K, et al.: CRIF1 overexpression facilitates tumor growth and metastasis through inducing ROS/NFκB pathway in hepatocellular carcinoma. Cell Death Dis 11: 332, 2020.
14. Chen X, Lu P, Wang DD, et al.: The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues. Gene 595: 221-226, 2016.
15. Zheng YJ, Zhao JY, Liang TS, et al.: Long noncoding RNA SMAD5-AS1 acts as a microRNA-106a-5p sponge to promote epithelial mesenchymal transition in nasopharyngeal carcinoma. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 33: 12915-12928, 2019.

16. Wu J, Huang J, Wang W, et al.: Long non-coding RNA Fer-1-like protein 4 acts as a tumor suppressor via miR-106a-5p and predicts good prognosis in hepatocellular carcinoma. Cancer Biomark 20: 55-65, 2017.
17. Yue B, Sun B, Liu C, et al.: Long non-coding RNA Fer-1-like protein 4 suppresses oncogenesis and exhibits prognostic value by associating with miR-106a-5p in colon cancer. Cancer science 106: 1323-1332, 2015.
18. Moreno CS: SOX4: The unappreciated oncogene. Semin Cancer Biol, 2019.
19. Hanieh H, Ahmed EA, Vishnubalaji R and Alajez NM: SOX4: Epigenetic regulation and role in tumorigenesis. Semin Cancer Biol, 2019.
20. Wei F, Jiang X, Gao HY and Gao SH: Liquiritin induces apoptosis and autophagy in cisplatin (DDP)-resistant gastric cancer cells in vitro and xenograft nude mice in vivo. Int J Oncol 51: 1383-1394, 2017.
21. Bhan A, Soleimani M and Mandal SS: Long Noncoding RNA and Cancer: A New Paradigm. Cancer Res 77: 3965-3981, 2017.
22. Liu Z, Sun M, Lu K, et al.: The long noncoding RNA HOTAIR contributes to cisplatin resistance of human lung adenocarcinoma cells via downregualtion of p21(WAF1/CIP1) expression. PLoS One 8: e77293, 2013.
23. Yan J, Dang Y, Liu S, Zhang Y and Zhang G: LncRNA HOTAIR promotes cisplatin resistance in gastric cancer by targeting miR-126 to activate the PI3K/AKT/MRP1 genes. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, 2016.
24. Shi L, Yin W, Zhang Z and Shi G: Down-regulation of miR-26b induces cisplatin resistance in nasopharyngeal carcinoma by repressing JAG1. FEBS open bio 6: 1211-1219, 2016.
25. Zhi F, Zhou G, Shao N, et al.: miR-106a-5p inhibits the proliferation and migration of astrocytoma cells and promotes apoptosis by targeting FASTK. PLoS One 8: e72390, 2013.
26. He QY, Wang GC, Zhang H, et al.: miR-106a-5p Suppresses the Proliferation, Migration, and Invasion of Osteosarcoma Cells by Targeting HMGA2. DNA and cell biology 35: 506-520, 2016.

Figures

Figure.1

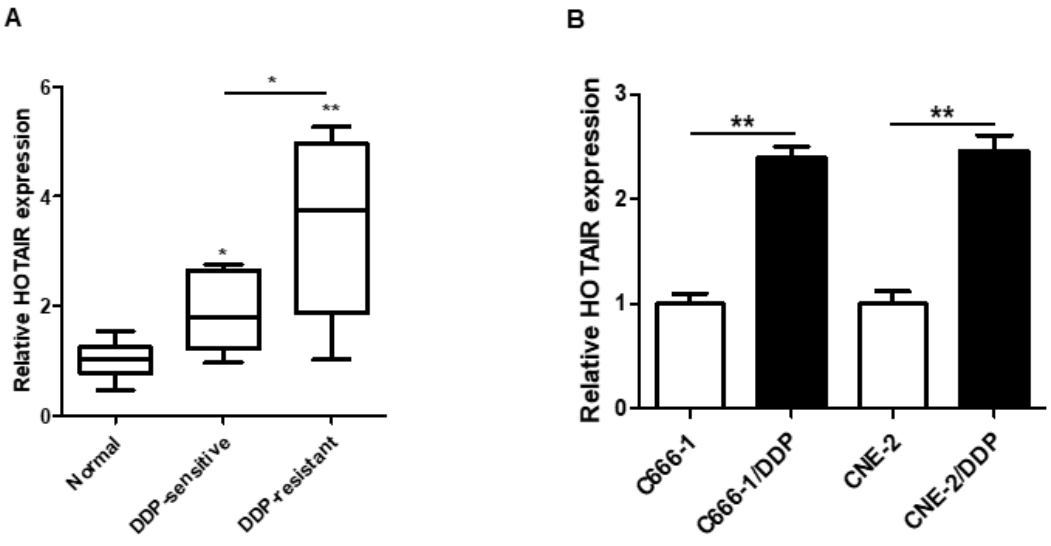

Figure 1

HOTAIR was upregulated in DDP-resistant NPC tissues and cells. The expression of HOTAIR in DDP-sensitive and DDP-resistant NPC tissues (A), C666-1/DDP and CNE2/DDP cells (B) and their matched controls were measured by qPCR. *p<0.05, **p<0.01.

Figure.2

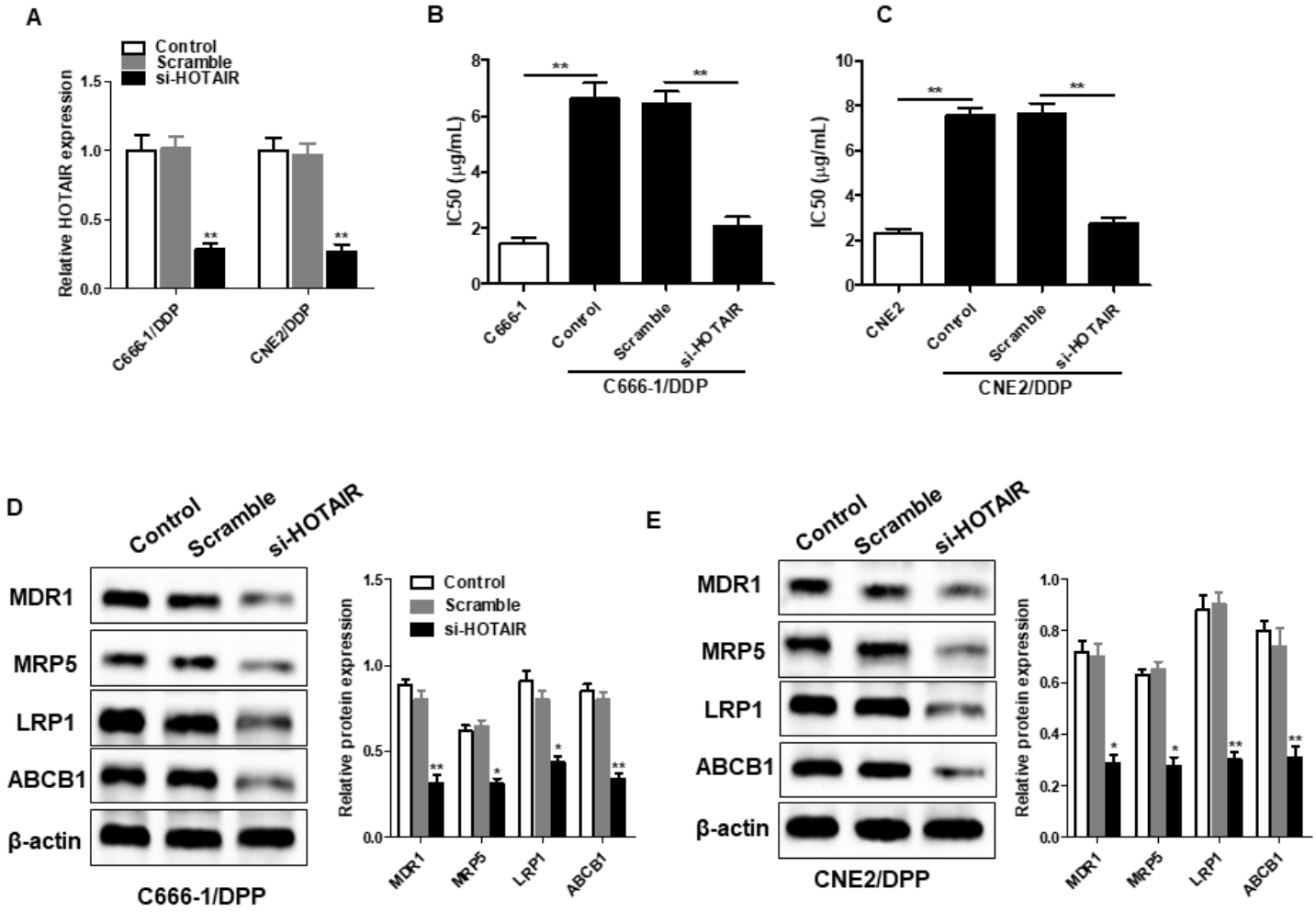

Figure 2

Down-regulation of HOTAIR decreased the resistance of C666-1/DDP and CNE2/DDP cells to DDP. HOTAIR siRNA was transfected into C666-1/DDP and CNE2/DDP cells, following transfection for 48 h, the interference efficiencies were detected with qPCR (A). The IC50 values of DDP (B, C), the protein levels of MDR1, MRP5, LRP1and ABCB1 (D, E) were detected by CCK-8 and Western blotting. *p<0.05, **p<0.01.

Figure.3

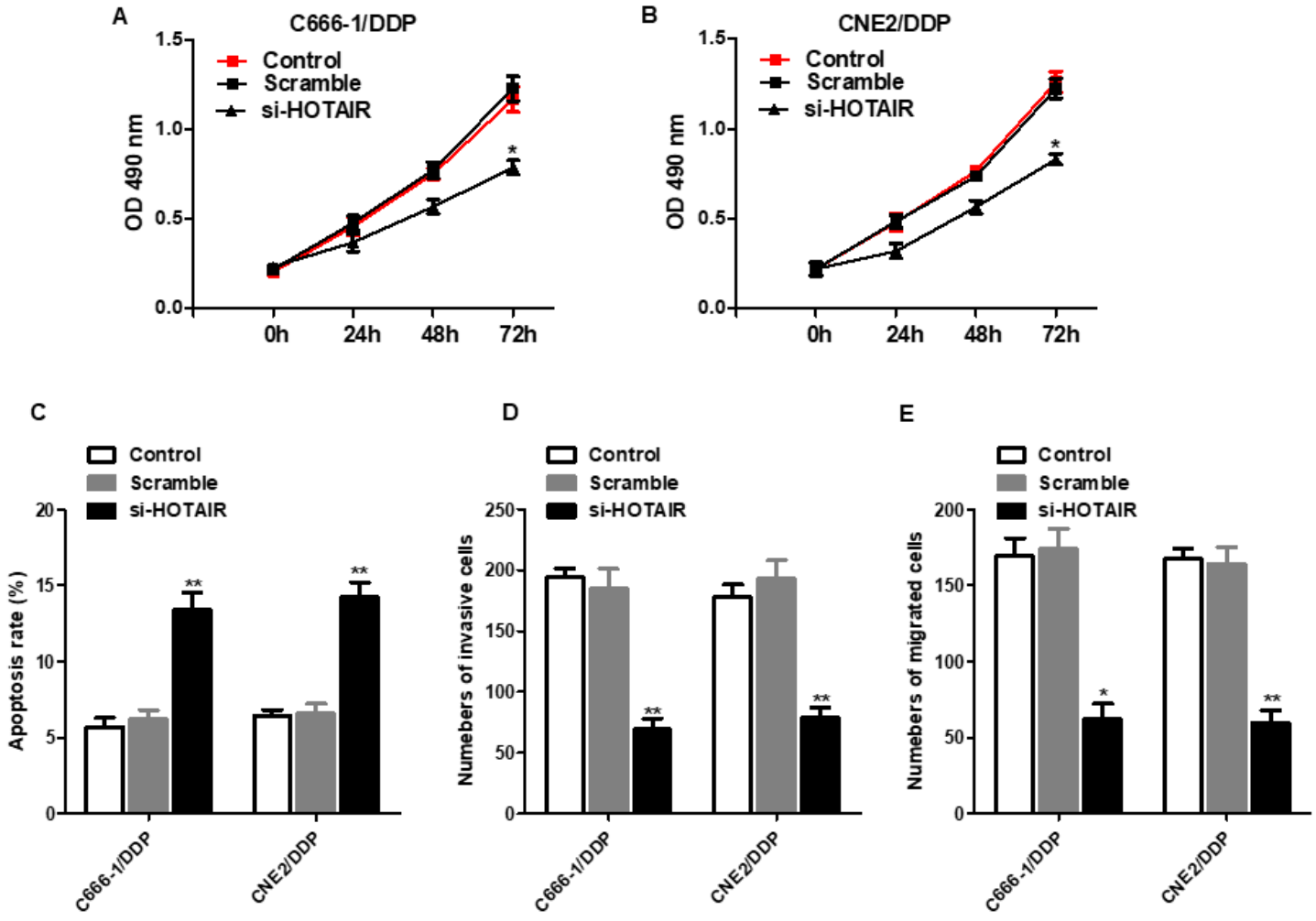

Figure 3

Interference with HOTAIR inhibited proliferation and invasion, and promoted apoptosis of C666-1/DDP and CNE2/DDP cells. HOTAIR siRNA was transfected into C666-1/DDP and CNE2/DDP cells, following transfection for 48 h, the cell proliferation (A, B), apoptosis (C), invasion and migration (D, E) were detected by CCK-8, AnnexinV/PI and Transwell *p<0.05, **p<0.01.

Figure.4

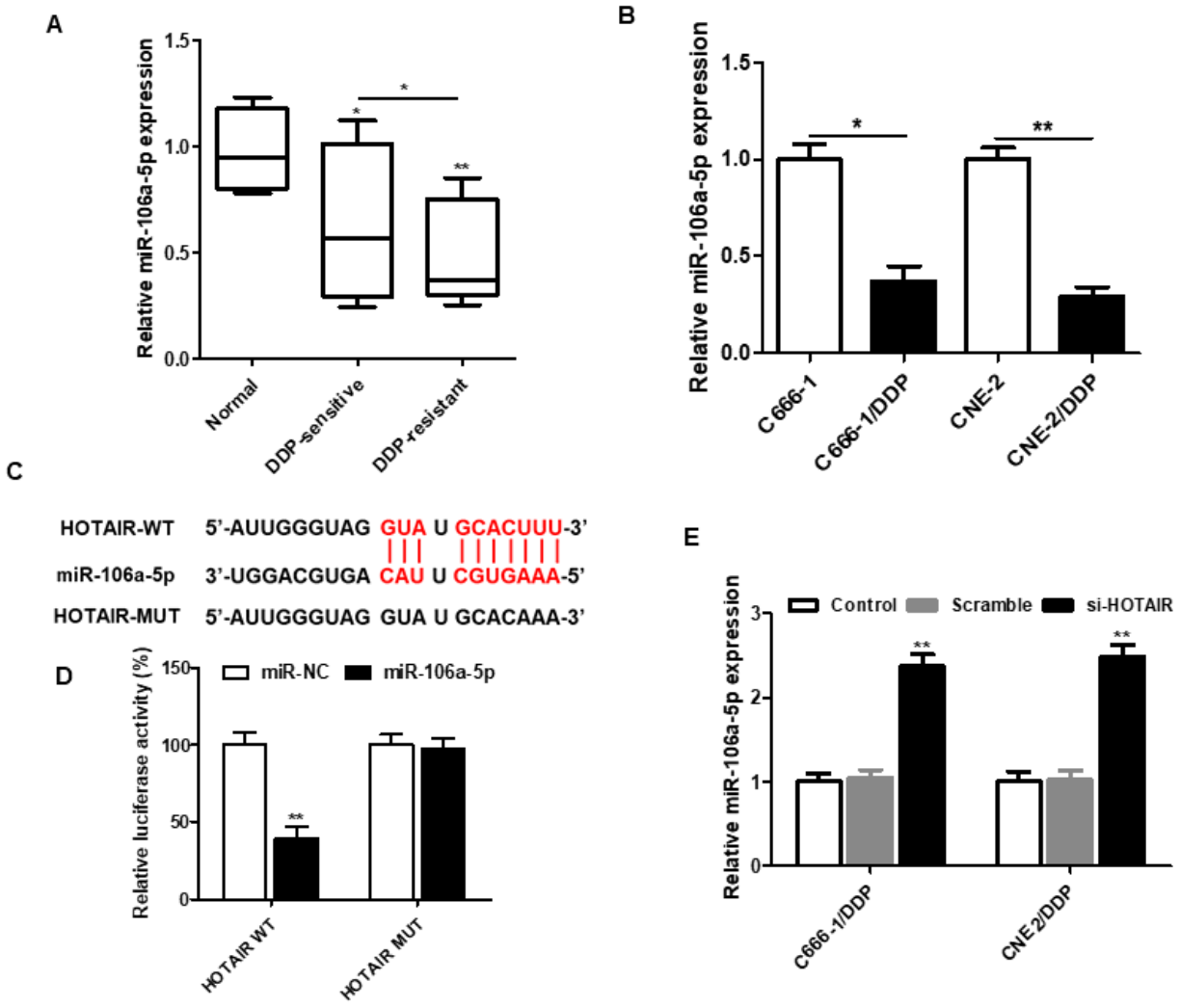

Figure 4

miR-106a-5p was downregulated in DDP-resistant NPC tissues and cells and regulated by HOTAIR. The expression of HOTAIR in DDP-sensitive and DDP-resistant NPC tissues (A), C666-1/DDP and CNE2/DDP cells and their matched controls were measured by qPCR (B). (C) The binding site of HOTAIR and miR-106a-5p predicted by StarBase v2.0. (D) The Luciferase activities of HOTAIR-WT (HOTAIR-MUT) reporters in C666-1/DDP and CNE2/DDP cells co-transfected with miR-106a-5p mimic or NPC mimic were assessed by Dual-Luciferase reporter assay. (E) HOTAIR siRNA was transfected into C666-1/DDP and CNE2/DDP cells, following transfection for 48 h, the expression of miR-106a-5p were detected by qPCR. *p<0.05, **p<0.01.

Figure.5

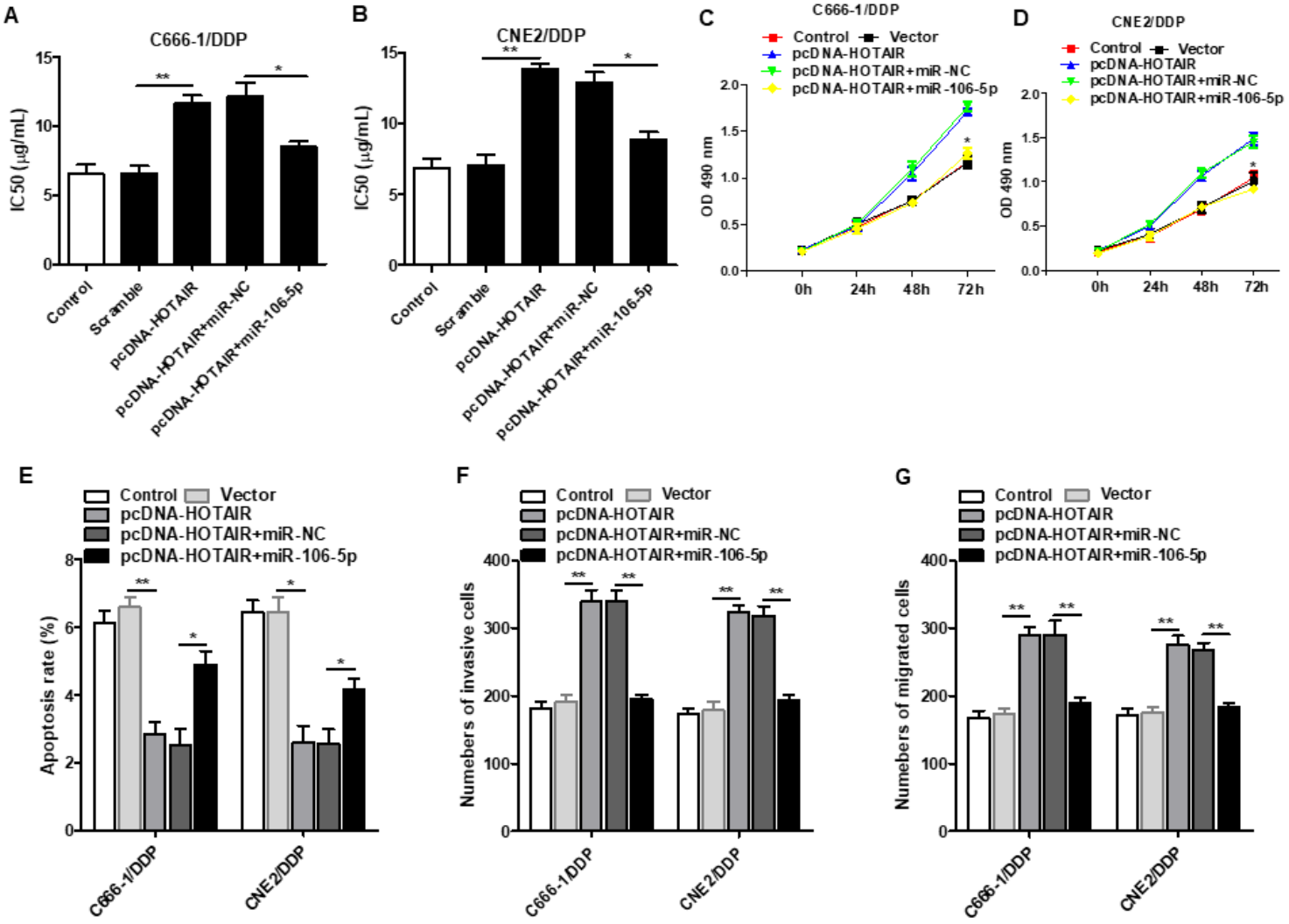

Figure 5

HOTAIR regulated the DDP resistance, cell proliferation, invasion and apoptosis of C666-1/DDP and CNE2/DDP cells by binding miR-106a-5p. HOTAIR overexpression vector was transfected into C666-1/DDP and CNE2/DDP cells alone or together with miR-106a-5p mimic, following transfection for 48 h, the IC50 values of DDP (A, B), the cell proliferation were detected by CCK-8 (C, D), and cell apoptosis (E), invasion (F) and migration (G) were detected by AnnexinV/PI and Transwell.*p<0.05, **p<0.01.

Figure.6

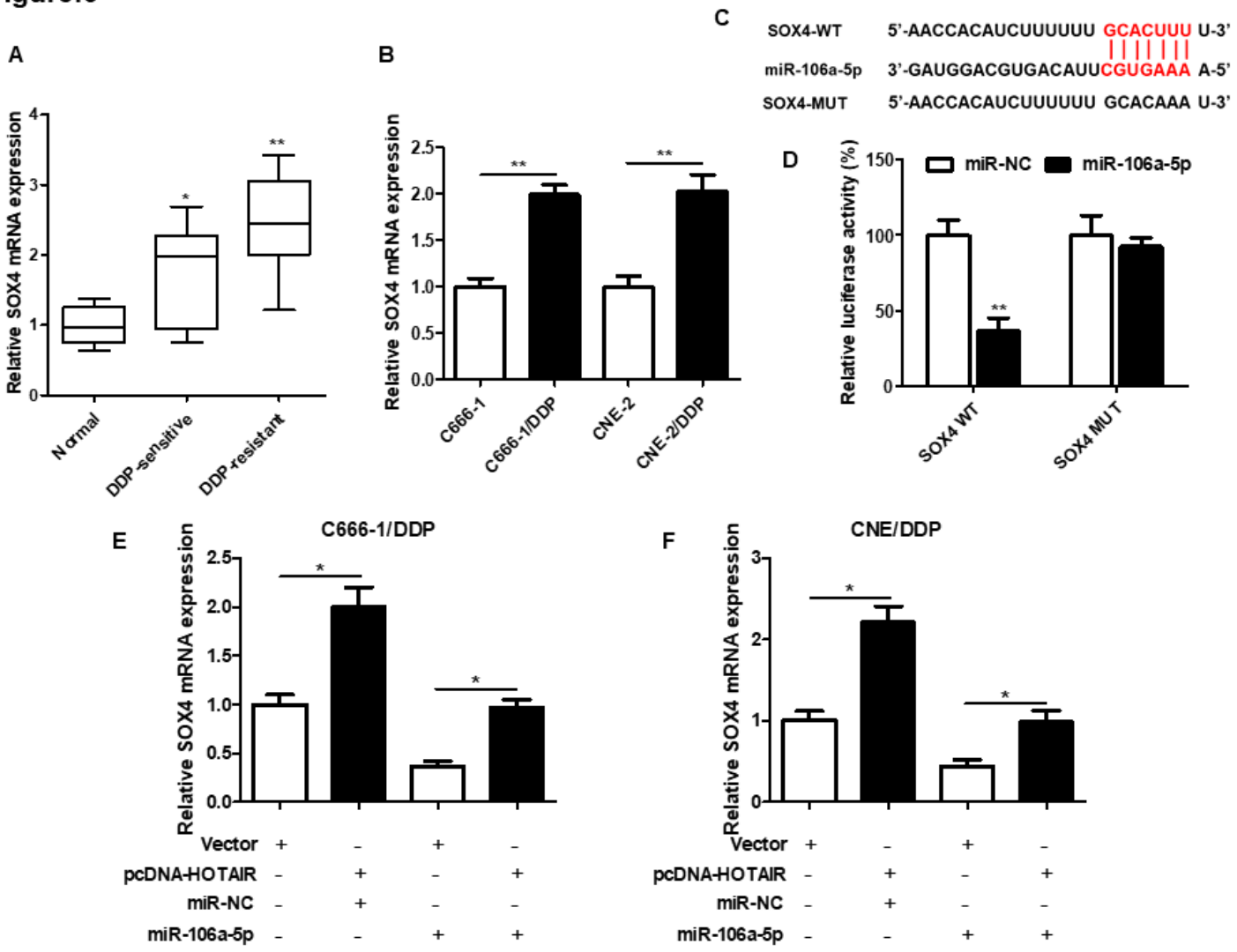

Figure 6

SOX4 was a target of miR-106a-5p. The expression of SOX4 in DDP-sensitive and DDP-resistant NPC tissues, C666-1/DDP and CNE2/DDP cells and their matched controls were measured by qPCR (A, B). Binding sites of miR-106a-5p and SOX4 predicted by TargetScan Human 7.2 (C). The Luciferase activities of SOX4-WT (SOX4-MUT) reporters in C666-1/DDP and CNE2/DDP cells transfected with miR-106a-5p mimic or NPC mimic were assessed by Dual-Luciferase reporter assay (D). HOTAIR overexpression vector and miR-106a-5p mimic were transfected respectively or co-transfected into C666-1/DDP and CNE2/DDP cells, and the expression of SOX4 was detected by qPCR (E, F). *p<0.05, **p<0.01.

Figure.7

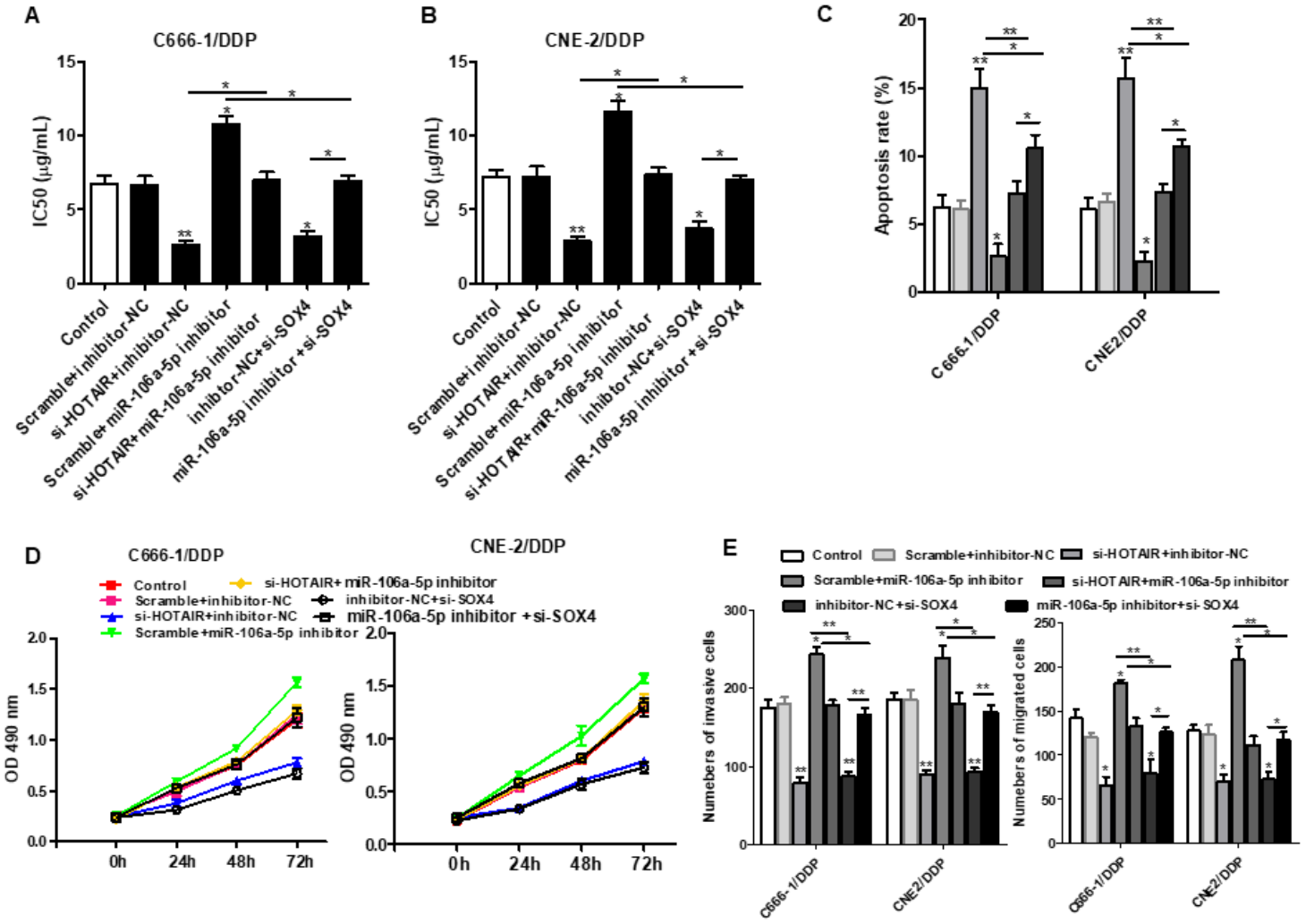

Figure 7

HOTAIR regulated the DDP resistance, apoptosis, cell proliferation, invasion and migration of C666-1/DDP and CNE2/DDP cells via miR-106a-5p/SOX4 axis. The HOTAIR siRNA, miR-106a-5p inhibitor, HOTAIR siRNA + miR-106a-5p inhibitor, SOX4 siRNA, miR-106a-5p inhibitor+ SOX4 siRNA were transfected into C666-1/DDP and CNE2/DDP cells, following transfection for 48 h, the IC50 values of DDP (A, B). The apoptosis was determined by AnnexinV/PI (C). Cell proliferation (D), invasion and migration (E) were detected by CCK-8 and Transwell. *p<0.05, **p<0.01.