HOTAIR is a potential target for the treatment of cisplatin-resistant ovarian cancer

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Received December 3, 2013; Accepted January 29, 2015

DOI: 10.3892/mmr.2015.3562

Abstract. Previous studies have demonstrated that the presence of Hox transcript antisense intergenic RNA (HOTAIR) is correlated with poor survival in several types of cancer, including breast cancer, and promotes tumor metastasis. Currently, little is known regarding the correlation between HOTAIR and chemoresistance in cancer. The current study aimed to investigate the role of HOTAIR in epithelial ovarian cancer, and the correlation between HOTAIR and cisplatin resistance. Reverse transcription-quantitative polymerase chain reaction was conducted to detect HOTAIR expression in the ovarian specimens and ovarian carcinoma cell lines. The results indicated that the expression level of HOTAIR was higher in epithelial ovarian cancer tissues than the level in the benign ovarian tissues. The expression level was also higher in late-stage malignant ovarian tumors compared with the level in early-stage tumors. Levels of HOTAIR were also higher in the SKOV-3CDDP/R cisplatin-resistant ovarian carcinoma cell line than in the SKOV-3 cisplatin-sensitive cell line. The knockdown of HOTAIR using siRNAs with transfection reagent suppressed cell proliferation, reduced the invasion ability of the cells and notably, it restored cisplatin-sensitivity of the cisplatin-resistant cells specifically by enhancing cisplatin-induced cytotoxicity and apoptosis in SKOV-3CDDP/R cells. In conclusion, HOTAIR may be used in the development of novel treatments for ovarian cancer, particularly those that are resistant to conventional therapies.

Introduction

Ovarian cancer accounts for 3% of cases of cancer in females and is the leading cause of gynecological malignancy-related mortality (1,2). Approximately 70% of patients with ovarian cancer are not diagnosed until the late stages (stages III and IV), due to the lack of characteristic symptoms and effective screening methods (3). Although histologically, ovarian cancer encompasses multiple different types, epithelial ovarian cancer (EOC) accounts for the majority of malignant ovarian tumors and can further be classified into eight distinct histological subtypes (4). The standard treatment for EOC is cytoreduction, with first-line platinum-based chemotherapy. Although EOC is highly responsive to the initial chemotherapy, the majority of patients experience relapse, due to the intrinsic and acquired resistance of the cancer cells to cytotoxic drugs, and the 5-year survival rate is <30% for patients in the advanced stages (5,6). Therefore, novel strategies that enhance sensitivity or reverse resistance to chemotherapy are urgently required for patients with EOC, particularly those that develop recurrent disease.

There are numerous non-protein-coding RNA (ncRNA) genes in the human genome. In contrast to other types of ncRNAs, such as microRNAs, whose roles have been greatly investigated, little is known regarding long ncRNAs (7,8). The various roles served by the long ncRNAs in malignant transformation and tumor growth are becoming increasingly recognized (9). Among these long ncRNAs exists one that was identified by a custom tilling array of the HOXC locus (12q13.13) (2), termed Hox transcript antisense intergenic RNA (HOTAIR).

HOTAIR has been observed to be overexpressed in several types of tumorous tissues, including breast, colorectal, hepatocellular, pancreatic and non-small cell lung cancer, compared with the levels in normal tissues. The high expression level has been observed to be correlated with increased cell invasiveness and enhanced cancer metastasis (10-14). A previous study has demonstrated that HOTAIR represses the transcription of specific genes by binding to the polycomb repressive complex (PRC)2, retargeting it to the locus and leading to H3K27me3 (10). Studies have suggested that HOTAIR-mediated suppression of genes in cancer is PRC2-dependent and PRC2-independent (10,13,15). Thus far, HOTAIR is recognized as a negative prognostic factor in various types of cancer, but the role HOTAIR serves, and its association with chemoresistance, remain to be investigated in EOC.

The aim of the present study was to investigate the role of HOTAIR in epithelial ovarian cancer and the correlation between HOTAIR and cisplatin resistance.

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Key words: Hox transcript antisense intergenic RNA, ovarian cancer, chemoresistance, invasion, long noncoding RNA

Materials and methods

Patient samples. A total of 80 freshly frozen ovarian specimens were obtained with informed consent from patients at the time of surgery, including 50 samples from patients with primary EOC and 30 samples of benign ovarian tissues for controls, obtained during hysterectomies for benign diseases. Surgery was performed at the Department of Obstetrics and Gynecology of the Second Affiliated Hospital of Harbin Medical University (Harbin, China) between October 2011 and November 2012. None of the participants had received chemotherapy prior to surgery. Patient data, including histopathological diagnosis, tumor grade and FIGO stages were obtained from medical records. The present study was approved by the ethics committee of Harbin Medical University.

Cells and reagents. The SKOV-3CDDP/R cisplatin-resistant epithelial ovarian cancer cell line and its parental variant SKOV-3 were purchased from the cell collection of the China Academy of Chinese Medical Sciences (Beijing, China). All cells were cultured in HyClone RPMI-1640 containing 10% HyClone fetal bovine serum medium (GE Healthcare Life Sciences, Logan, UT, USA) with 1% penicillin and streptomycin (Genom Biotech Pvt., Ltd., Shanghai, China) in a humidified 5% CO_2 atmosphere at 37˚C. Cisplatin was purchased from Beyotime Institute of Biotechnology (Shanghai, China) and administered to cells at doses of 0, 2.5, 5, 10 and 20 μg/ml.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from frozen tissues or cell lines using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was synthesized using Oligo$(dT)_{15}$ primers with M-MLV Reverse Transcriptase (Promega Corporation, Madison, WI, USA). HOTAIR expression levels were evaluated using qPCR with the AccuPower 2X GreenStar master mix solution (Bioneer Corporation, Daejeon, Korea) in a StepOnePlus Real-Time PCR system (Applied Biosystems Life Technologies, Foster City, CA, USA). The primer sequences for HOTAIR and β-actin (internal control) were as follows: Forward: 5'-TGGGGAACTCTGACTCGC-3' and reverse: 5'-TCGCCGCCGTCTGTAACT-3' for HOTAIR; and forward: 5'-GTCAGGTCATCACTATCGGCAAT-3' and reverse: 5'-AGAGGTCTTTACGGATGTCAACGT-3' for β-actin. The reaction conditions were as follows: 5 min at 94˚C, followed by 40 cycles of 94˚C for 30 sec, 56˚C for 30 sec and 72˚C for 30 sec. The melting curves for the two genes were analyzed to confirm the purity of the amplified products. Samples were analyzed in triplicate in three independent experiments. HOTAIR expression values were normalized to β-actin. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Transfection of siRNA. siRNA oligonucleotides (50 nM) were transfected into the two types of cells with Lipofectamine 2000 transfection reagent (Invitrogen Life Technologies) according to the manufacturer's instructions. The transfection solution was removed after 6 h of incubation and replaced with fresh growth medium. After 48-h incubation, the cells were assayed for the expression level of HOTAIR post-transfection. An siRNA targeting HOTAIR and a negative control (NC) siRNA (silencer NC siRNA) were purchased from Bioneer Corporation. The sequences of the siRNAs were as follows: siHOTAIR: 5'-UCAGUGUCAGAAAAUGCUU-3' and siNC: 5'-CCUACGCCAAUUUCGU-3'.

Analysis of cell viability and apoptosis. WST-8 dye from a Cell Counting kit-8 (CCK-8; Beyotime Institute of Biotechnology) was used to detect cell viability according to the manufacturer's instructions. Cell staining was conducted with an Annexin V-FITC Apoptosis Detection kit, including propidium iodide (BD Biosciences, San Jose, CA, USA). Staining was quantified by flow cytometry analysis using a FACSCalibur cell sorting system (BD Biosciences). Cells in the early and late stages of apoptosis were evaluated. Samples were analyzed in triplicate in three independent experiments.

Analysis of cell invasiveness. A cell-invasion assay was conducted using a Costar 24-well Transwell chamber with a pore size of 8 μm (Corning Life Sciences, Corning, NY, USA), and the upper membrane surfaces were coated with 30 μl Matrigel (1:2 dilution; BD Biosciences). Cells ($1x10^5$) were seeded in the compartment chamber in serum-free medium subsequent to RNA interference. The lower compartment was filled with cell culture medium (RPMI-1640; HyClone) supplemented with 10% FBS. After 24 h, cells on the upper membrane surface were removed and the invaded cells on the bottom surface were fixed with methanol and stained with 1% crystal violet. The invading cells were examined, counted and images were captured using digital microscopy (P6000; Nikon Corporation, Tokyo, Japan). Four fields were counted per filter in each chamber.

Statistical analysis. The differences in the continuous data between two groups were analyzed with the independent t-test and results expressed as the mean ± standard deviation. The analysis of HOTAIR expression levels in the different groups of ovarian tissues was performed using nonparametric tests (Mann-Whitney U test or Kruskal-Wallis H test). $P<0.05$ was considered to indicate a statistically significant difference. Each experiment was conducted at least three times in triplicate. Statistical analyses were performed with SPSS software, version 16.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Expression of HOTAIR in clinical ovarian tissues. In the current study, RT-qPCR was performed in order to define the expression levels of HOTAIR in the clinical ovarian tissues. Initially, the tissues were divided into two groups: The benign ovarian (BO) and EOC tissues (Fig. 1A). The results indicated that the HOTAIR expression level was significantly higher in the EOC group compared with the BO group ($P<001$). Next, the HOTAIR levels in the 50 cases of primary malignant ovarian tissues were compared. The tissues were divided into groups according to tumor subtype, tumor grade and tumor stage. The observed differences in expression levels between the different tumor subtypes or tumor grade were not significant ($P=0.466$ and 0.342, respectively; Fig. 1B and C). However, a significant difference in expression levels was identified

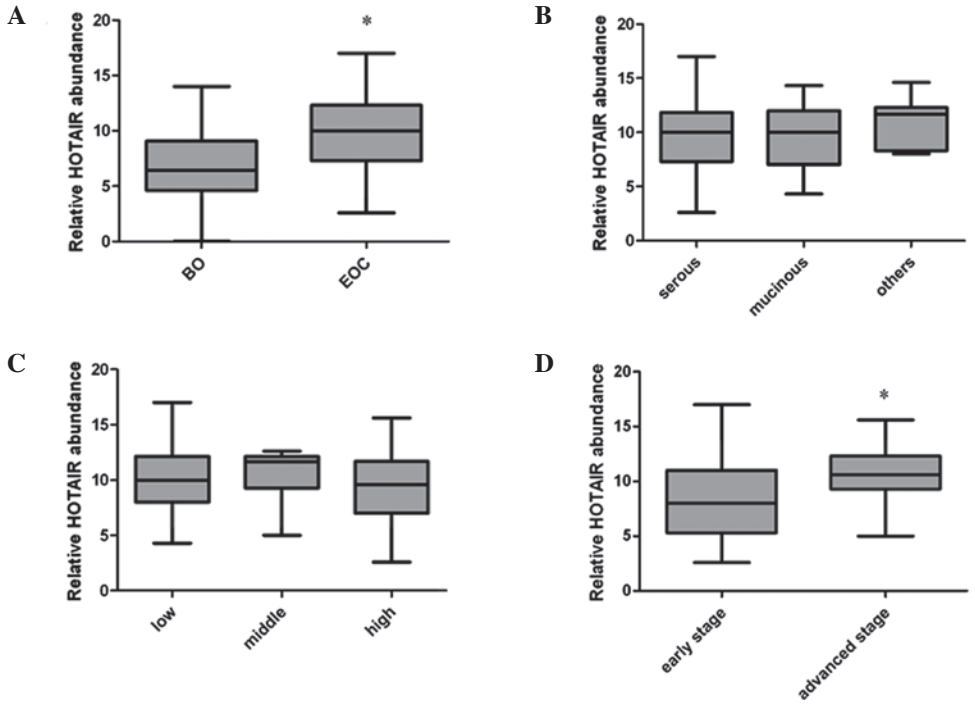

Figure 1. HOTAIR expression in ovarian tissues and its clinical significance. (A) Differences in HOTAIR expression levels between EOC (n=50) and BO (n=30) samples. *P<0.05 vs. BO tissues; Mann-Whitney U test. (B) HOTAIR expression levels in different tumor subtypes, including serous, mucinous and others (endometrioid, clear cell and undifferentiated EOC) (C) HOTAIR expression levels in various tumor grades. (D) HOTAIR expression levels in early (n=15) and advanced (n=35) stage EOC samples (early stage, FIGO stages I and II; advanced stage, FIGO stages III and IV). *P<0.05 vs. early stage; Mann-Whitney U test. The results are presented as a log2 scale. The expression of HOTAIR was normalized to β-actin with respect to specimen BO. HOTAIR, Hox transcript antisense intergenic RNA; BO, benign ovarian; EOC, eptihelial ovarian carcinoma.

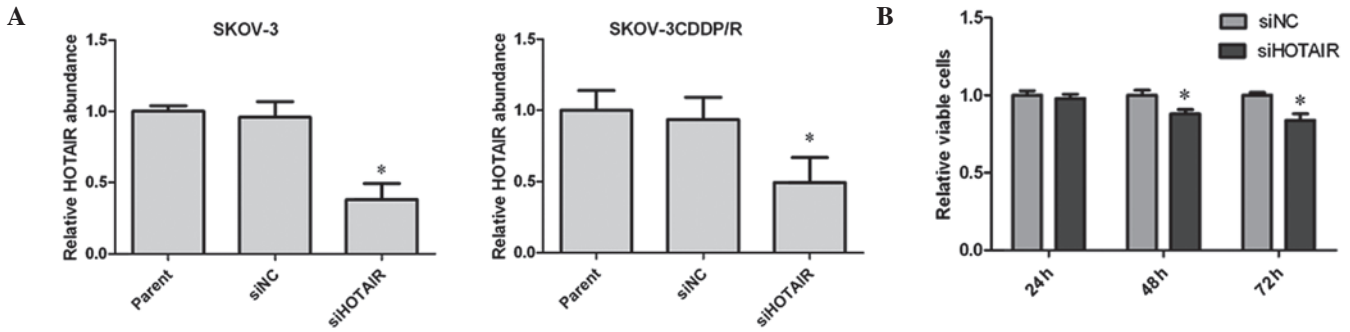

Figure 2. Effective knockdown of HOTAIR suppresses cell viability in SKOV-3 cells (A) Effective knockdown of HOTAIR in SKOV-3 epithelial ovarian cancer cells and their cisplatin-resistant equivalents (SKOV-3/CDDP/R cells) 48 h after siRNA treatment. *P<0.05 vs. the parent cells; t-test. (B) Effects of transfection with siNC or siHOTAIR on SKOV-3 cell viability at different time points. *P<0.05, **P<0.01 vs. siNC; t-test. HOTAIR, Hox transcript antisense intergenic RNA; siNC, negative control siRNA.

between samples from the early and advanced stages of EOC (P=0.027), the HOTAIR expression level in stage III and IV EOC was significantly higher than that of the stage I and II EOC samples (Fig. 1D).

Knockdown of HOTAIR suppresses cell proliferation in SKOV-3 cells. To determine the effects of the HOTAIR expression level on EOC cells, an RNA interference assay was used to modulate the expression of HOTAIR. As presented in Fig. 2A, the endogenous HOTAIR levels determined by RT-qPCR in the two cell lines were effectively reduced by siHOTAIR treatment, but not by siNC treatment. Next, the cell viability was assessed using the CCK-8 assay kit at different time points following transfection in SKOV-3 cells. The results indicated that the effective knockdown of HOTAIR significantly inhibited cell proliferation at 48 and 72 h (Fig. 2B).

Cisplatin-induced cytotoxicity in SKOV-3 and SKOV-3CDDP/R cell lines. In order to examine the association of HOTAIR expression patterns with drug sensitivity, the cisplatin-resistant cell line SKOV-3CDDP/R and its parental cell line SKOV-3 were selected. The CCK-8 assay was employed to compare the effects of cisplatin on the proliferation of the SKOV-3 and SKOV-3CDDP/R cells. A range of doses (0-20 μg/ml) of cisplatin were administered to the cells for 24 h. The results indicated that the viabilities of the SKOV-3 and SKOV-3CDDP/R cells were reduced by cisplatin in a dose-dependent manner (Fig. 3A). Additionally, SKOV-3CDDP/R cells exhibited

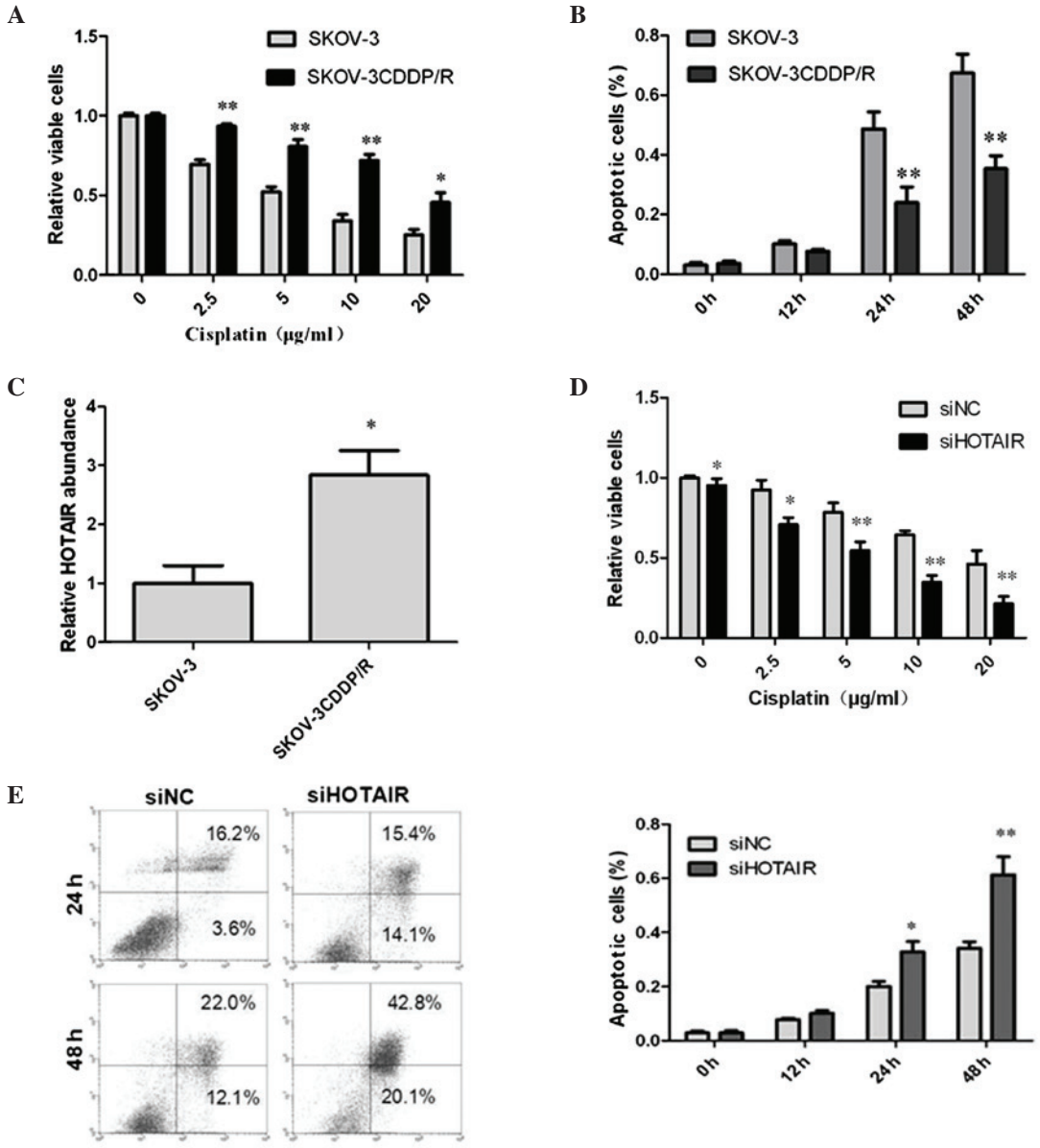

Figure 3. Effects of HOTAIR knockdown on ovarian carcinoma cells. All data are presented as the mean ± standard error, based on at least three independent experiments. (A) Dose-response cell viability for SKOV-3 and SKOV-3CDDP/D cells following treatment with cisplatin for 24 h. *P<0.05 and **P<0.01 vs. SKOV-3 cells; t-test. (B) Cisplatin-induced apoptosis of SKOV-3 and SKOV-3CDDP/D cells following treatment with cisplatin (5 μg/ml) at different time points. *P<0.05 and **P<0.01 vs. SKOV-3 cells; t-test. (C) Relative expression levels of HOTAIR in SKOV-3 and SKOV-3CDDP/R cell lines. *P<0.05 vs SKOV-3 cells; t-test. (D) Dose-response cell viability of SKOV-3CDDP/R cells following treatment with different doses of cisplatin for 24 h after transfection with siNC or siHOTAIR. *P<0.05 and **P<0.01 vs. siNC; t-test. (E) Cisplatin-induced apoptosis following transfection of SKOV-3CDDP/R cells with cisplatin (5 μg/ml) at different time points. The right image shows the percentage of apoptotic cells following transfection of SKOV-3CDDP/R cells with siNC or siHOTAIR at different time points. The left image shows representative images of apoptosis. *P<0.05 and **P<0.01 vs. siNC; t-test. All graphs are representative of at least three independent experiments. HOTAIR, Hox transcript antisense intergenic RNA; siNC, negative control siRNA.

greater resistance to cisplatin than the SKOV-3 cells. As evaluated using the IC50 values, SKOV-3 cells exhibited a reduction of ~4-fold in viability compared with SKOV-3CDDP/R cells (P<0.05). The greatest difference in cisplatin-induced cytotoxicity between the two types of cells was observed when cells were exposed to 5 μg/ml cisplatin (P<0.001). This result verifies the chemosensitivity of the two cell lines; cisplatin resistance in SKOV-3CDDP/R cells and cisplatin sensitivity in the parental SKOV-3 cells. In order to further assess the cisplatin sensitivity, flow cytometry was used to define cisplatin-induced apoptosis in the cells with 5 μg/ml cisplatin at different time points (Fig 3B). The results demonstrated that there was a greater level of cisplatin-induced apoptosis in the SKOV-3 cells compared with the SKOV-3CDDP/R cells.

HOTAIR is more abundant in resistant cells than in cisplatin-sensitive cells. The HOTAIR expression level was examined by qPCR in the paired cell lines. There was a significant difference in the levels of HOTAIR expression between the two cell lines (Fig. 3C). The expression level of HOTAIR in SKOV-3CDDP/R cells was significantly greater than that in SKOV-3 cells (>2.5 fold) indicating that HOTAIR may be a factor in the reduction of chemosensitivity in SKOV-3CDDP/R cells.

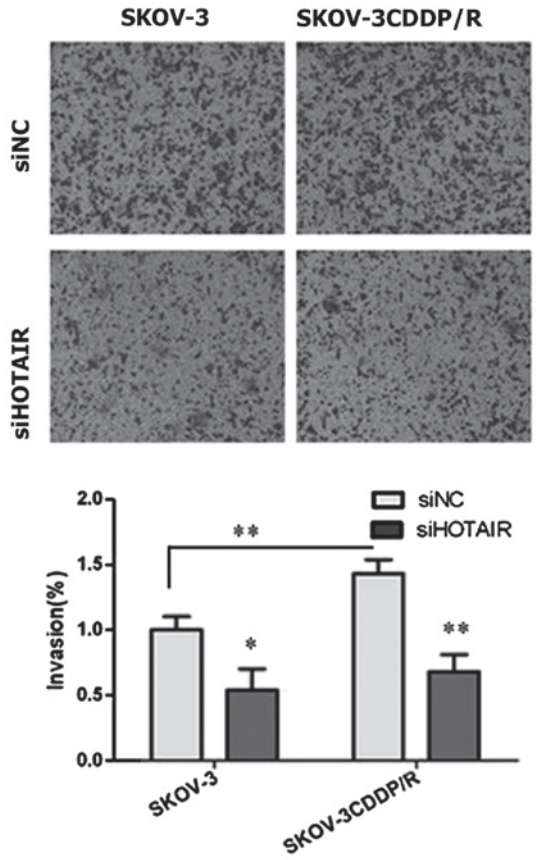

Figure 4. Selective knockdown of HOTAIR reduced the invasion ability of ovarian carcinoma cells. The columns represent the relative number of invaded cells. *P<0.05 and **P<0.01 vs. siNC; t-test. The relative level of cell migration is presented as the mean ± standard error, based on at least three independent experiments. The images are representative of the invasiveness of the two cell lines prior and subsequent to transfection. Magnification, x100. HOTAIR, Hox transcript antisense intergenic RNA; siNC, negative control siRNA.

Knockdown of HOTAIR restores the cisplatin sensitivity of cisplatin-resistant cells. The siRNAs were utilized to investigate the effects of RNA interference on the cisplatin sensitivity of the SKOV-3CDDP/R cisplatin-resistant cell line. Following the transient transfection of siRNAs, the cells were treated with various doses of cisplatin (0-20 μg/ml). After 24 h, the CCK-8 assay was applied to assess the cell viability as in the prior assay. For the groups of cells exposed to 0 μg/ml cisplatin for 24 h, siHOTAIR-treated cells displayed a significant difference in cell viability compared with siNC treated cells (P=0.48). However, with greater concentrations of cisplatin, the difference in the cell viability distinctly increased between the two groups. These results demonstrate that the cells treated with siHOTAIR were more sensitive to cisplatin than the cells treated with siNC (Fig 3D). It is clear that the knockdown of HOTAIR suppressed levels of cell proliferation and more notably resensitized the SKOV-3CDDP/R cells to cisplatin. To further investigate the role of HOTAIR in drug sensitivity, flow cytometry was used to define cisplatin-induced apoptosis following transfection of SKOV-3CDDP/R cells with cisplatin (5 μg/ml) at different time points (Fig 3E). The results indicated that the level of apoptosis was higher in the HOTAIR knockdown group compared with the negative control group, and the differences increased in a time-dependent manner. Hence, the alteration of HOTAIR expression levels in ovarian carcinoma cells markedly alters cisplatin-induced cytotoxicity and susceptibility to apoptosis.

Knockdown of HOTAIR reduces the invasion ability of ovarian carcinoma cells. Next, the invasiveness of the cells was assessed by a Transwell invasion assay following the knockdown of HOTAIR (Fig. 4). The invasion capacities were markedly reduced when HOTAIR expression was knocked down *in vitro* using siRNAs in the SKOV-3 (P=0.035) and SKOV-3CDDP/R (P<0.01) cells, as indicated by the reduced number of cells invading through the Matrigel-coated membrane. Additionally, the cisplatin-resistant SKOV-3CDDP/R cells that exhibited higher levels of HOTAIR expression displayed a stronger ability for invasion compared with the parental counterpart (P<0.01).

Discussion

Previous studies have demonstrated that HOTAIR is overexpressed in several types of tumor tissues at a greater level than in corresponding non-tumor tissues. One study also indicated that HOTAIR is highly expressed in aggressive and invasive pancreatic tumors (10-13). To the best of our knowledge, the current study demonstrated for the first time that the expression levels of HOTAIR in ovarian tissues are also associated with the malignant phenotype (P<0.001). The differences in expression levels at different tumor stages were also analyzed. The results demonstrated that there was a significant difference in the levels of HOTAIR in early and late stage malignant ovarian tumors. The stage of a tumor is associated with the recurrence of ovarian cancer, as advanced diseases present with a greater rate of recurrence within two years (16). The current *in vitro* study has also indicated that HOTAIR knockdown inhibits cell proliferation (P<0.05), suggesting that HOTAIR promotes tumor progression and is associated with tumor recurrence.

To further investigate the function of HOTAIR in ovarian cancer recurrence, it is necessary to begin with chemoresistance. The recurrence of ovarian cancer is mainly attributed to the chemoresistance of tumor cells to conventional chemotherapy, which may be due to reduced drug accumulation, increased levels of glutathione and metallothionein and enhanced DNA repair (17-19). In the present study, the HOTAIR level was demonstrated to be increased in the SKOV-3CDDP/R cisplatin-resistant cell line compared with the SKOV-3 cisplatin-sensitive cell line. These two cell lines have similar genetic backgrounds with minor variations as the SKOV-3CDDP/R cisplatin-resistant cell line was established by repeated *in vitro* treatment of SKOV-3 cells with a clinically relevant dose of cisplatin (20). For this reason they were superior to other cell lines and were selected as paired models for an *in vitro* investigation of drug resistance. The silencing of HOTAIR by siRNA alone did not markedly reduce the cell viability (reduced by <10%). However, once the two groups were treated with cisplatin, the siHOTAIR group demonstrated enhanced sensitivity to cisplatin indicated by significantly reduced cell viability and an elevated rate of apoptosis. The SKOV-3CDDP/R cells were resensitized to cisplatin subsequent to the knockdown of HOTAIR. This indicates that HOTAIR may not only promote tumor proliferation, but also

can strengthen the effects of antitumor drugs. This result suggests that elevated HOTAIR expression may be responsible for drug resistance in patients with recurrent ovarian cancer and the interference of HOTAIR may restore this sensitivity. In addition, the preoperative quantification of HOTAIR in tumor biopsies or ascitic fluid may aid in predicting the tumor chemosensitivity. Although the present study observed that the knockdown of HOTAIR resensitized SKOV-3CDDP/R cells by inhibiting cisplatin-induced apoptosis (Fig. 3E), further studies are required to explore the influence of HOTAIR expression levels on the chemosensitivity of ovarian cancer and associated tumor recurrence. The microarray analysis between primary and recurrent ovarian cancer may aid the investigation of the underlying molecular mechanisms.

The present study also demonstrated that the selective knockdown of HOTAIR markedly weakens the invasion capacity of the two cell lines. The cisplatin-resistant cells with higher HOTAIR expression presented higher invasion ability than their counterparts, indicating that the HOTAIR expression level is positively correlated to the invasion capability of ovarian carcinoma cells with similar genetic backgrounds. This result further verifies the fact that HOTAIR is a notable factor associated with tumor invasion, and it is important in the development of metastases.

Though ovarian cancer has been well-investigated, the pathogenesis of ovarian cancer remains unclear. There are multiple theories involving the pathogenesis of ovarian cancer; the upsurging cancer stem cell (CSC) hypothesis is attracting an increasing level of attention. According to this theory, cancer is a mixed aberrant hierarchical organization of the non-tumorigenic progeny of CSCs and the tumorigenic CSCs, which have the stem-like ability of self-renewal and are able to generate heterogeneous lineages of cells (21,22). This CSC hypothesis can also be applied to recurrent ovarian cancer growth, since the stem-like properties enable the cancer to select for more aggressive and chemoresistant cells subsequent to chemo- and radiotherapy (23,24). HOTAIR regulates chromatin silencing on the HOXD locus by retargeting the PRC2, which contains three subunits termed EZH2, EED and SUZ12. EZH2 is reported to be an essential regulator of embryonic and adult stem cell differentiation, and SUZ12 is also required for embryonic stem cell differentiation (25-27). There are also studies indicating that HOTAIR is required for the maintenance of stemness in cancer cells lines, involving EMT triggering (28). Future studies may focus on the role of HOTAIR in CSCs in order to assess whether the HOTAIR-mediated retargeting of PRC2 is responsible for the differentiation of CSCs.

In conclusion, HOTAIR serves important roles in ovarian cancer progression, including increasing cell proliferation and promoting cell invasion. The examination of HOTAIR levels in tumors has the potential to be used to complement to diagnosis, as a negative factor in prognosis and as a predictor for the risk of recurrence.

References

1. Jemal A, Thomas A, Murray T and Thun M: Cancer statistics. CA Cancer J Clin 52: 23-47, 2002.
2. Bertone-Johnson ER: Epidemiology of ovarian cancer: a status report. Lancet 365: 101-102, 2005.
3. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ: Cancer statistics, 2008. CA Cancer J Clin 58: 71-96, 2008.
4. Bell DA: Origins and molecular pathology of ovarian cancer. Mod Pathol 18 (Suppl 2): S19-S32, 2005.
5. Sood AK and Buller RE: Drug resistance in ovarian cancer: from the laboratory to the clinic. Obstet Gynecol 92: 312-319, 1998.
6. McGuire WP, Hoskins WJ, Brady MF, *et al*: Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 334: 1-6, 1996.
7. Mattick JS: The genetic signatures of noncoding RNAs. PLoS Genet 5: e1000459, 2009.
8. Yasuda J and Hayashizaki Y: The RNA continent. Adv Cancer Res 99: 77-112, 2008.
9. Ponting CP, Oliver PL and Reik W: Evolution and functions of long noncoding RNAs. Cell 136: 629-641, 2009.
10. Gupta RA, Shah N, Wang KC, Kim J, *et al*: Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464: 1071-1076, 2010.
11. Yang Z, Zhou L, Wu LM, *et al*: Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann Surg Oncol 18: 1243-1250, 2011.
12. Kogo R, Shimamura T, Mimori K, Kawahara K, *et al*: Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 71: 6320-6326, 2011.
13. Kim K, Jutooru I, Chadalapaka G, *et al*: HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene 32: 1616-1625, 2013.
14. Liu XH, Liu ZL, Sun M, Liu J, Wang ZX and De W: The long non-coding RNA HOTAIR indicates a poor prognosis and promotes metastasis in non-small cell lung cancer. BMC Cancer 13: 464, 2013.
15. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, *et al*: Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 129: 1311-1323, 2007.
16. Gadducci A, Cosio S, Zola P, *et al*: Surveillance procedures for patients treated for epithelial ovarian cancer: a review of the literature. Int J Gynecol Cancer 17: 21-31, 2007.
17. Godwin AK, Meister A, O'Dwyer PJ, Huang CS, Hamilton TC and Anderson ME: High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc Natl Acad Sci USA 89: 3070-3074, 1992.
18. Kelley SL, Basu A, Teicher BA, Hacker MP, Hamer DH and Lazo J: Overexpression of metallothionein confers resistance to anticancer drugs. Science 241: 1813-1815, 1998.
19. Parker RJ, Eastman A, Bostick-Bruton F and Reed E: Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. J Clin Invest 87: 772-777, 1991.
20. Stordal BK, Davey MW and Davey RA: Oxaliplatin induces drug resistance more rapidly than cisplatin in H69 small cell lung cancer cells. Cancer Chemother Pharmacol 58: 256-265, 2006.
21. Pardal R, Clarke MF and Morrison SJ: Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 3: 895-902, 2003.
22. Curley MD, Garrett LA, Schorge JO, Foster R and Rueda BR: Evidence for cancer stem cells contributing to the pathogenesis of ovarian cancer. Front Biosci (Landmark Ed) 16: 368-392, 2011.
23. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, *et al*: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 7; 444: 756-760, 2006.
24. Neuzil J, Stantic M, Zobalova R, Chladova J, Wang X, *et al*: Tumour-initiating cells vs. cancer 'stem' cells and CD133: what's in the name? Biochem Biophys Res Commun 355: 855-859, 2007.
25. Pasini D, Bracken AP, Hansen JB, Capillo M and Helin K: The polycomb group protein Suz12 is required for embryonic stem cell differentiation. Mol Cell Biol 27: 3769-3779, 2007.
26. Chen YH, Hung MC and Li LY: EZH2: a pivotal regulator for controlling cell differentiation. Am J Transl Res 4: 364-375, 2012.
27. Khalil AM, Guttman M, Huarte M, Garber M, *et al*: Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci USA 106: 11667-11672, 2009.
28. Pádua Alves C, Fonseca AS, Muys BR, *et al*: Brief report: The lincRNA Hotair is required for epithelial-to-mesenchymal transition and stemness maintenance of cancer cells lines. Stem Cells 31: 2827-2832, 2013.