Original Article
Mir-200c inhibits HOTAIR expression resulting in the decrease of chemoresistance in ovarian cancer stem cells

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Abstract: In this study, we investigated whether miR-200c overexpression would increase the sensitivity of epithelial ovarian cancer (EOC) stem cells (CSCs) to chemotherapy drugs through down-regulation of lncRNA HOTAIR. We used a magnetic-activated cell sorting system to isolate the SKOV3 CD44+CD117+CSCs from the selected human EOC SKOV3 cells that were stably transduced with lentivirus miR-200c. HOTAIR, a direct target of miR-200c, was validated by using the wild-type and the mutant region HOTAIR luciferase reporters. The results showed the overexpression of miR-200c in SKOV3 CD44+CD117+CSCs significantly decreased the drug resistant to paclitaxel and cisplatin compared with SKOV3 CD44+CD117+CSCs transduced with the lentivirus-mock or the wild-type of SKOV3 CD44+CD117+CSCs. Moreover, SKOV3 CD44+CD117+CSCs with miR-200c overexpression dramatically reduced its metastatic potential from the tumor tissues to the nude mouse lungs in contrast to SKOV3 CD44+CD117+CSCs without miR-200c overexpression. The direct down-regulation of HOTAIR was miR-200c dependent because luciferase reporter and rescue assay results showed that the putative miR-200c-binding site has the inhibitory effect on HOTAIR expression. Collectively, the increased sensitivity of SKOV3 CD44+CD117+CSCs to paclitaxel or cisplatin may be modulated by overexpression of miR-200c that directly inhibits HOTAIR expression.

Keywords: Epithelial ovarian cancer, cancer stem cells, drug resistance, miR-200c, lincRNA HOTAIR

Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynaecological cancer with the majority of patients succumbing to chemotherapy-resistant disease. Currently, there are no specific or sensitive clinical biomarkers that maybe implemented to identify chemoresistance and give insight to prognosis. Thus, understanding the related mechanisms of chemotherapy resistance and how it can be prevented or reversed is a pivotal challenge in the treatment of EOC in the worldwide [4, 32, 33].

Accumulating studies have demonstrated that there are a subpopulation of cells that are associated with properties of stem/progenitor-like cells known as cancer stem cells (CSCs) in drug-resistant EOC tissues, and CSCs are believed to be 'seed cell' in cancer-initiating potential, invasion, metastasis, and resistance to traditional chemotherapy [5, 13, 22]. The existence of EOC CSCs might explain why standard chemotherapy may shrink most tumors, however, left less highly lethal malignancy regrow and eventually cause a relapse. Therefore, it is necessary to investigate the molecular mechanisms of EOC CSC chemoresistance and to find out the new strategies for overcoming chemoresistance of CSCs in EOC patients [1, 2].

Emerging evidence supports the view that epigenetically regulation function appears to play a crucial role in the development of inherent and acquired chemoresistance in EOC. Aberrant

epigenetic states can be reversed by drug therapy, and thus maintenance and regulation of epigenetic change is a potential target to halt or reverse chemotherapeutic resistance [4, 5]. Indeed, there have been reports that the microRNAs (miRNAs) have emerged as potential therapeutic candidates by virtue of their ability to down-regulation of EOC therapeutic resistance. Some studies have demonstrated that epithelial-mesenchymal transition (EMT) has an established role in promoting tumor progression and the acquisition of therapeutic resistance [11, 12, 23]. For example, in cisplatin-resistant ovarian cancer tissues and cell lines, the EMT phenotype was correlated with decreased miR-186 expression, increased Twist1 expression, chemoresistance, and poor prognosis in EOC patients. While introducing miR-186 into EOC cells led to a reduction in Twist1 expression along with mesenchymal-to-epithelial transition, decrease of chemoresistance, and enhanced cell apoptosis [32]. In another study, miR-9 was expressed significantly higher in drug-sensitive patients than in drug-resistant ones, and inhibition of miR-9 resulted in decreased clonal formation and sensitivity to cisplatin, suggesting high expression of miR-9 was associated with enhanced tumorigenesis and increased sensitivity of the tumor cells to cisplatin treatment in primary ovarian tumor cells [31]. Our previous study demonstrated that the miR-200c overexpression, by modulating the EMT, specifically inhibited the zinc-finger E-box binding homeobox 1 expression in the SKOV3 CD117+CD44+CSCs, and reduced cell tumorigenicity in nude mouse model [5].

In addition, long intervening non-coding RNA (lincRNA) HOTAIR is well studied among lncRNA and has been shown to have an important functions in normal and cancer cells. Dysregulated HOTAIR correlates highly with tumor invasion and metastasis [17]. Our study showed that the downregulated HOTAIR expression in SKOV3 CD117+CD44+CSCs significantly decreased the tumor growth and lung metastasis in xenograft mice [25]. However, how to co-regulate the properties of EOC SKOV3 CD117+CD44+CSCs between the miR-200c and the HOTAIR remains a little known. To this end, we investigated the interaction between the miR-200c and the HOTAIR, and wanted to know how to modulate the resistance to chemotherapy drugs in EOC

SKOV3 CD117+CD44+CSCs by miR-200c direct down-regulation of HOTAIR.

Materials and methods

Cell line

SKOV3 cell line, a well-established ovarian cancer model system from ovarian cancer patient, was ordered from the Cellular Institute in Shanghai, China. Cells were cultured in complete media consisting of RPMI 1640, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum. The medium was refreshed every 3 days to maintain adherent cells. When reached 90% confluence, cells were harvested with 0.25% trypsin -1 mM EDTA (Sigma-Aldrich, St. Louis, MO, USA).

Isolation of human SKOV-3 CD44+CD117+ CSCs, transduction of lentivirus miR-200c and production of stable expression colonies

CD44+CD117+cells were sorted from the SKOV-3 cell line by using the magnetic-activated cell sorting (MACS, Miltenyi Biotec., Bergisch Gladbach, Germany) as described in the previous published papers [5, 15]. Briefly, CD44+cells were isolated by using mouse antihuman CD44 antibody coupled to magnetic microbeads, and followed by the magnetic column selection or depletion (Miltenyi Biotec., Bergisch Gladbach, Germany). Resulting cells were then depleted of CD117-cells by using mouse antihuman CD-117 antibody coupled to magnetic microbeads (Miltenyi Biotec., Bergisch Gladbach, Germany), and we named CD44+CD117+cells for EOC cancer stem cells (EOC SKOV-3 CD44+CD117+CSCs). To generate the miR-200c expression lentivirus vector, we amplified an insert (full-length mouse miR-200c) from SKOV-3 cell line by PCR. The lentivirus miR-200c was produced from the transient transduction of the HEK293T cells with pHAGE-CMV-miR-200c-IzsGreen, psPAX2, and pMD2.G plasmid DNAs plus Lipofectamine 2000 (Invitrogen, USA), respectively, according to the manufacturer's protocol. Forty-eight hours after the co-transfection, the lentivirus-bearing supernatants were collected and passed through a 0.45-mm filter. SKOV-3 cells were infected with the pHAGE-CMV-miR-200c-IzsGreen lentivirus, and were selected by the IzsGreen expression [10]. The stable expression colonies were selected by limiting the dilution assay [19], and then SKOV-3 CD44+

CD117+CSCs were sorted from the SKOV-3 cells with stably infected lentivirus miR-200c by using above-mentioned MACS.

Cytotoxicity assay in a various CD44+CD117+CSCs

Cytotoxicity assay was used to test the ability of paclitaxel and cisplatin (Sigma-Aldrich, Missouri, USA) to induce cell death. $3×10^4$ CD44+ CD117+CSCs transduced with lentivirus-miR-200c were resuspended in 96-plates, and then added at concentrations of 10 μg/ml, 20 μg/ml, 30 μg/ml, 40 μg/ml, 50 μg/ml, 60 μg/ml, and 70 μg/ml paclitaxel respectively, or 0.5 μg/ml, 1.0 μg/ml, 1.50 μg/ml, 2,0 μg/ml, 2.5 μg/ml, 3.0 μg/ml, 3.5 μg/ml, 4.0 μg/ml, 4.5 μg/ml, and 5.0 μg/ml cisplatin, respectively; cells were incubated for 72 h. Each concertration were done repeat three experiments. As a control, the CD44+CD117+CSCs transduced with lentivirus-mock and the wild type of CD44+ CD117+CSCs were used in this assay. Chemotherapeutic sensitivity was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetry (MTT) assay. The OD values were read at dual wave lengths of 450 nm and 630 nm to determine cell viability by using a microplate reader (Thermo Fisher Labsystems) [5, 30].

RNA extraction and quantitative real-time reverse transcription-PCR (qRT-PCR)

Total RNA from the different cultured cells and mouse tumor tissues was isolated using a Qiagen RNeasy Kit (Qiagen, Valencia, CA, USA) following by manufacturer's protocol. Single-stranded cDNA was prepared using SuperScript III reverse transcriptase (Invitrogen). qRT-PCR was carried out on an ABI step one plus real-time system (Applied Biosystems, USA). cDNAs were amplified by PCR with primers as follows: HOTAIR: sense, 5'-GGTAGAAAAAGCAACC ACG-AAGC-3'; antisense, 5'-TTGGGGAAGCATTTTCTG-AC-3'; β-actin (sense, 5'-GGACTTC GAGCAAGA-GATGG-3'; antisense, 5'-AGCACTGTGTTGGCGT-ACAG-3'). U6-RT Primer, 5'-GTCGTATCCAGTGCA-GGGTCCGAGGTATTCGCACTGGATACGACAAA-TATGGAAC-3'; sense, 5'-TGCGGGTGCTCGCTTC-GGCAGC-3'; URP (Universal Reverse Primer), 5'-CCGG CAGGGTCCGAGGT-3'; TGF-b1: sense, 5'-TGGAAACCCACAACGAAATCT-3'; antisense, 5'-GCTGAGGTATCGCCAGGAAT-3'; Bmi-1: sense, 5'-TGCTGATGCTGCCAATGG-3'; antisense, 5'-TTAC-

TTTCCGATCCAATCTGTTCTG-3'. The mRNA levels of the interested genes were expressed as the ratio of each gene of interest to β-actin or U6 mRNA for each sample. At least 3 biologic replicates and triplicate PCRs were used to calculate relative expression. The relative mRNA or miRNA levels were calculated using the comparative cycle threshold (C_t) method ($\Delta\Delta C_t$). Briefly, the C_t values for the normalization gene were subtracted from C_t values of the target gene to achieve the ΔC_t value. The $2^{-\Delta}C_t$ was calculated for each sample, and then each of the values was divided by a control sample to achieve the relative mRNA or miRNA levels ($\Delta\Delta C_t$) [17, 26].

Immunofluorescence detection of metastatic tumor cells in mouse lung tissues

At the end of the experiments, lung tissues were removed from the xenograft mice, fixed in 10% formalin, and then embedded in paraffin. Lung tissue sections of 4 μm thin were cut and mounted on SuperFrost Plus glass slides; the Luciferase positive cells were visualized using the immunofluorescence microscope at a magnification of ×200 [5, 16].

Double-luciferase reporter assay

The wild/mutated HOTAIR (NCBI: NR_0037-16.3, site from 978 to1008 nt) was generated by PCR from human genomic DNA. These DNA fragments were cloned into the pGL3-control plasmid (Promega, Madison, WI, USA), while pGL-3.0 (firefly luciferase) was used as an internal control. Briefly, HEK-293 or SKOV3 cells were seeded in 24 well cell culture clusters (Corning Incorporated; Corning, NY, USA). When reached 70% confluences, cells were cotransfected with the reporter constructs and hsa-miR-200c mimics for 36 h. Then the luciferase reporter assay was performed using a Double-Luciferase Assay system (Promega) per the manufacturer's instructions [9, 21].

Statistical analysis

Statistical analysis was performed using the Student's t-test for the difference between the experimental group and the control group. Data are expressed as mean ± standard error. Results for all analysis with a *P* value <0.05 indicate the statistically significant differences.
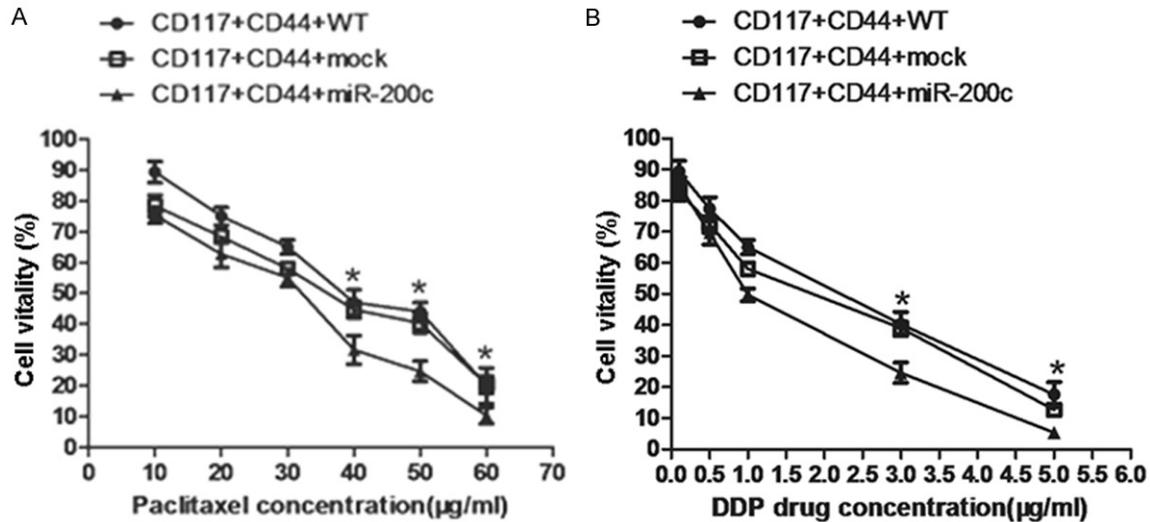
Figure 1. Cell vitality changes in SKOV3 CD44+CD117+CSCs infected with lentivirus-miR-200c. Overexpression of miR-200c in SKOV3 CD44+CD117+CSCs decreased the drug resistance to paclitaxel and cisplatin, respectively in vitro, which resulted in decrease of cell vitality when drug concentration was gradually increaed. A and B represent the cell vitality in a various cells to paclitaxel and cisplatin.

Results

miR-200c increases chemotherapeutic sensitivity in SKOV3 CD44+CD117+CSCs

To examine whether the overexpression of miR-200c in SKOV3 CD44+CD117+CSCs would decrease the resistance to chemotherapetic drugs, we assessed the sensitivity changes of SKOV3 CD44+CD117+CSCs to chemotherapetic drugs paclitaxel and cisplatin respectively; these drugs are commonly used in clinic EOC patient chemotherapy. The results showed the forced overexpression of miR-200c in SKOV3 CD44+CD117+CSCs decreased the resistance to all the two drugs in vitro compared with the control cells. It was found that the cell vitality of lentivirus-miR-200c transducted SKOV3 CD44+CD117+CSCs to paclitaxel was statistically significant decreased compared with the lentivirus-mock transducted SKOV3 CD44+CD117+CSCs (31% vs 45%, *P<0.05) and the SKOV3 CD44+CD117+CSCs without miR-200c transduction (31% vs 49%, *P<0.05), respectively when cells were incubated with paclitaxel (40 μg/ml) for 72 hours. As this concentration was increased by 60 μg/ml, the cell vitality was simultaneously decreased in the lentivirus-miR-200c transducted SKOV3 CD44+CD117+CSCs (10%), which was statistically significant compared with the lentivirus-mock transducted SKOV3 CD44+CD117+CSCs (20%, *P<0.05),

and the wild type of SKOV3 CD44+CD117+CSCs (21%, *P<0.05), respectively (**Figure 1A**). Similarly, the resistance of the lentivirus-miR-200c transducted SKOV3 CD44+CD117+CSCs to cisplatin was statistically significant lower than that of the lentivirus-mock transducted SKOV3 CD44+CD117+CSCs (25% vs 41%, *P< 0.05) and the wild type of SKOV3 CD44+ CD117+CSCs (25% vs 40%, *P<0.05) when cells were incubated with cisplatin (3.0 μg/ml) for 72 hours. As predicted, an inhibitory effect of cisplatin on above various cells was significantly increased when cisplatin concentration was reached by 60 μg/ml as is shown in **Figure 1B**. The findings implied that the sensitivity of these various treated SKOV3 CD44+CD117+ CSCs to paclitaxel and cisplatin was mainly depended on the miR-200c overexpression or not.

miR-200c decreases the expression of TGF-β1 and Bmi-1 in SKOV3 CD44+CD117+CSCs as well as cellular metastasis potential in mice

Next, we wanted to know whether decreased chemoresistance to drugs in lentivirus-miR-200c transducted SKOV3 CD44+CD117+CSCs was correlated with decreased expression of EMT promoter facror TGF-β1 and stem-like factor Bmi-1. The results of qRT-PCR in **Figure 2A**, **2B** showed the forced overexpression of miR-200c in SKOV3 cells (A) and in the tumor tis-
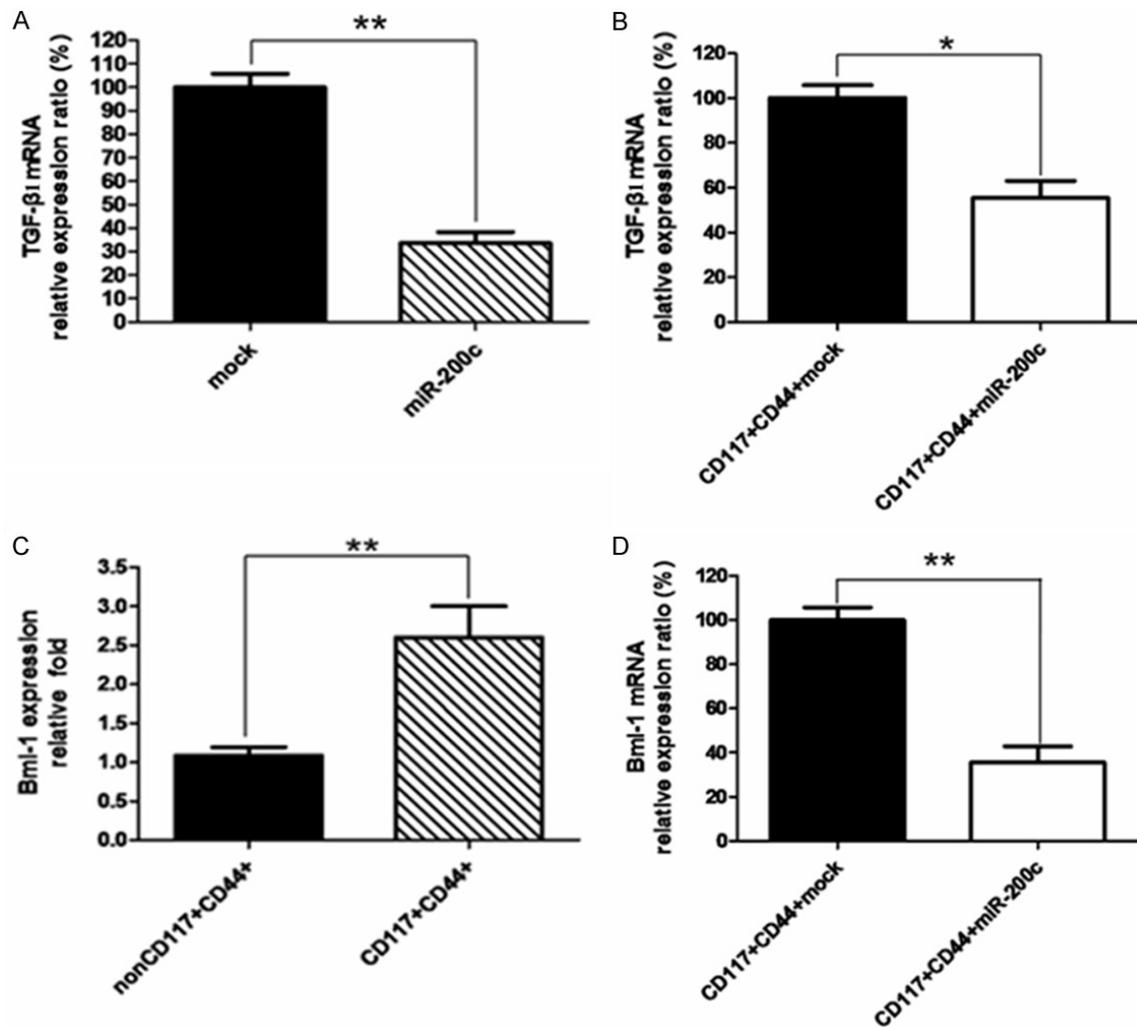
Figure 2. Relative expression of TGF-β1 and Bmi-1. Relative expression of TGF-β1 and Bmi-1 in SKOV3 cells and SKOV3 tumor tissues in nude mice was analyzed by real-time PCR. A, B represent the TGF-β1 expression in the lentivirus-miR-200c transducted or lentivirus-mock transducted SKOV3 cells (A), and in the tumor tissues from the nude mice injected with lentivirus-miR-200c transducted SKOV3 CD44+CD117+CSCs and lentivirus-mock transducted SKOV3 CD44+CD117+CSCs, respectively (B). C indicates the Bmi-1 relative expression differences in the lentivirus-miR-200c transducted SKOV3 non CD44+CD117+CSCs or SKOV3 CD44+CD117+CSCs. D shows the relative expression of Bmi-1 in the lentivirus-miR-200c transducted CD44+CD117+CSCs and lentivirus-mock transducted SKOV3 CD44+CD117+CSCs, respectively. *P<0.05, and **P<0.01.

sues from the nude mice injected with lentivirus-miR-200c transducted SKOV3 CD44+CD117+CSCs (B) significantly reduced the TGF-β1 expression in contrast to the lentivirus-mock transducted SKOV3 CD44+CD117+CSCs, which was statistically significant (**P<0.01, SKOV3 cells) or (*P<0.05, tumor tissues). Consistently, the Bmi-1 expression was 2.5 times higher in SKOV3 CD44+CD117+CSCs than that in SKOV3 non CD44+CD117+CSCs tested by qRT-PCR, which was also statistically significant (**P< 0.01) as is shown in **Figure 2C**. In addition, the Bmi-1 expression was markedly reduced in the

tumor tissues from the nude mice injected with the lentivirus-miR-200c transducted SKOV3 CD44+CD117+CSCs compared to the nude mice injected with the lentivirus-mock transducted SKOV3 CD44+CD117+CSCs (**Figure 2D**), and the difference was statistically significant (**P< 0.01).

Since CSCs have many features that distinguish them from mature, differentiated cells [1, 14], of which one particularly intriguing feature is that CSCs have a distant metastasis potential. To this end, we further tested the migrating
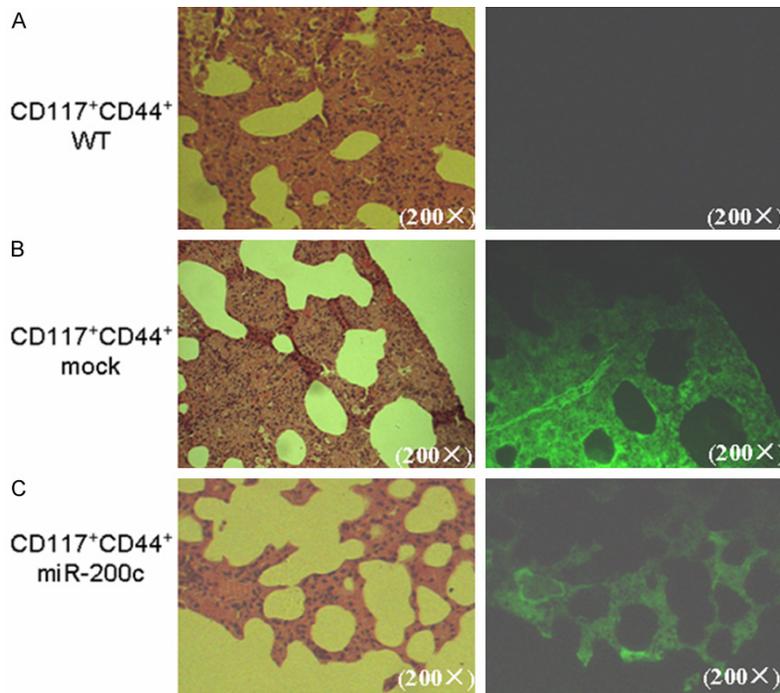
Figure 3. Images of tumor metastatic lung tissues from the nude mice. At the end of the experiments, lung tissues were removed from the nude mice subcutaneously injected with 5×10⁴ SKOV3 CD44⁺CD117⁺CSCs with lentivirus miR-200c infection, SKOV3 CD44⁺CD117⁺CSCs with lentivirus mock infection, and SKOV3 CD44⁺CD117⁺CSCs without lentivirus infection, respectively. Lung tissue sections were made and visualized by using a light microscope or an immunofluorescence microscope at a magnification of ×200. The left and the right side images were seen under a light microscope and immunofluorescence microscope in order. A-C represent the lung tissues from the nude mice injected with 5×10⁴ SKOV3 CD44⁺CD117⁺CSCs without lentivirus infection (A), with lentivirus mock infection (B), and with lentivirus miR-200c infection (C), respectively. These images suggested that less metastatic tumor cells were found in lung tissues that was mainly depended on the miR-200c overexpression or not.

capability of SKOV3 CD44⁺CD117⁺CSCs. **Figure 3** gives the images of tumor metastatic lung tissues from the nude mice. The left side images show three kinds of different treated SKOV3 CD44⁺CD117⁺CSCs in mouse lung tissues, and no green fluorescence was found in common light microscope. The right side image (middle) shows remarkably stronger green fluorescence in lentivirus-mock transducted SKOV3 CD44⁺CD117⁺CSCs than that in lentivirus-miR-200c transducted SKOV3 CD44⁺CD117⁺CSCs (bottom), suggesting a lot of lentivirus-mock transducted SKOV3 CD44⁺CD117⁺CSCs migrated to the lung tissues but only a few of miR-200c overexpressed SKOV3 CD44⁺CD117⁺CSCs did migration. The top image in right side does not indicate green fluorescence this is because the wild type of SKOV3 CD44⁺CD117⁺CSCs did not

receive the lentivirus construct transduction. From these results, we may conclude that the miR-200c overexpression in SKOV3 CD44⁺CD117⁺CSCs inhibited the TGF-β1 and the Bmi-1 expression as well as SKOV3 CD44⁺CD117⁺CSC lung metastatic ability in nude mice.

miR-200c binds HOTAIR and suppresses its expression

To understand the mechanisms underlying the suppressive effects of miR-200c on the SKOV3 CD117⁺CD44⁺CSC's chemoresistance and metastatic potential, we used three open-target prediction programs (TargetScan, picTar, and miRnada) to predict the targets of miR-200c. HOTAIR is a potential one of miR-200c modulation. The HOTAIR mRNA contained 9 basic continuous base complementary sites for the seed region of mature miR-200c (**Figure 4A**). The dual-luciferase reporter assay result exhibited that the luciferase activity of the reporter containing the wild-type of HOTAIR gene was significantly decreased following treatment with miR-200c mimics (80% vs 100%, *P<0.05), which suggested that the direct down-regulation of HOTAIR was miR-200c-dependent because the mutation in the putative miR-200c-binding sites had rescued the inhibitory effect as is shown in **Figure 4B**. In this study, we meanwhile predicted the targets of common precursor miR-200c-5p since we constructed the lentivirus-miR-200c precursor that may generate two common precursors (miR-200c-3c and miR-200c-5p) when the precursor was transducted to SKOV3 cells. HOTAIR mRNA contained 8 continuous base complementary sites for the seed region of miR-200c-5p precursor (**Figure 4C**). The luciferase activity of the reporter containing the HOTAIR gene's
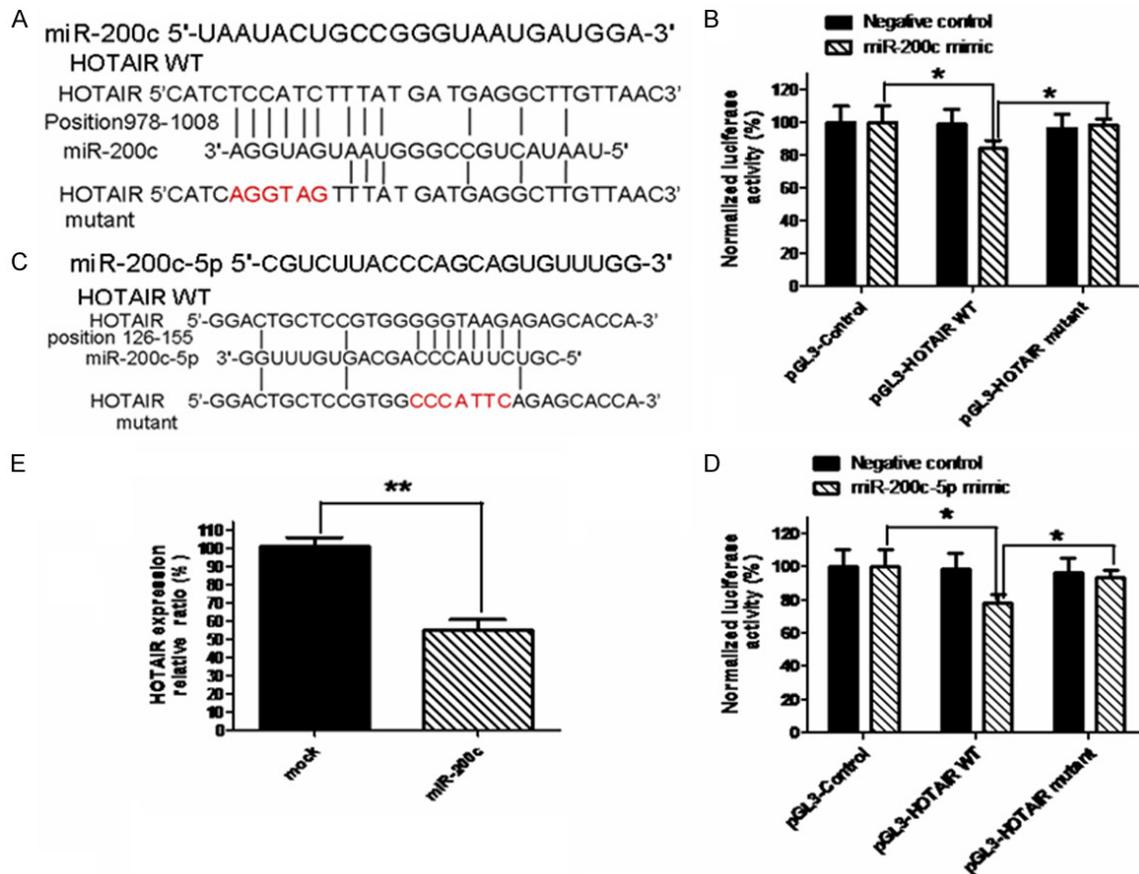
Figure 4. miR-200c targets HOTAIR. A shows miR-200c binding sequences in HOTAIR. The scarlet letters in the HOTAIR sequences show the gene mutations. B indicates that HT-293 cells in 96-well plates were cotransfected with the reporter constructs and hsa-miR-200c mimics for 36 h. 3'-UTR reporter activity was measured by a luciferase assay and normalized to the activity of renilla luciferase. C represents the common precursor miR-200c-5p binding sites in HOTAIR gene sequences. D shows HT-293 cells were cotransfected with the reporter constructs and hsa-miR-200c-5p mimics for 36 h, and the luciferase activity was measured. E exhibits the HOTAIR expression in lentivirus-miR-200c or lentivirus-mock stably infected SKOV3 cells measured by qRT-PCR. *$P<0.05$ and **$P<0.01$, referring to the differences as indicated.

wild-type was remarkably reduced after cotrasfection with miR-200c mimics (78% vs 100%, *$P<0.05$). Similarly, the mutation in the putative miR-200c-5p binding sites has rescued the inhibitory effect on HOTAIR expression (*$P<0.05$) as is shown in **Figure 4D**. These results suggested the miR-200c mimics did directly suppress the HOTAIR gene expression.

To further confirm whether HOTAIR is the direct target gene for miR-200c, we used qRT-PCR to test the changes of HOTAIR expression in response to forced miR-200c overexpression in SKOV3 cells. **Figure 4E** gives the HOTAIR expression was significant decreased in lentivirus-miR-200c stably transduced SKOV3 cells compared with in the lentivirus-mock stably

transducted SKOV3 cells (**$P<0.01$). This positive data was further confirmed by HOTAIR expression in tumor tissues from the nude mice injected with SKOV3 CD117$^+$CD44$^+$CSCs with lentivirus-miR-200c or lentivirus-mock, indicating the HOTAIR expression markedly reduced by 34% in tumor tissues from the nude mice injected with lentivirus-miR-200c transduced SKOV3 CD44$^+$CD117$^+$CSCs compared with the nude mice injected with lentivirus-mock transducted SKOV3 non-CD44$^+$CD117$^+$CSCs (**$P<0.01$), which was shown in **Figure 5**.

Discussion

The paclitaxel used in combination with cisplatin offers the first-line treatment option in
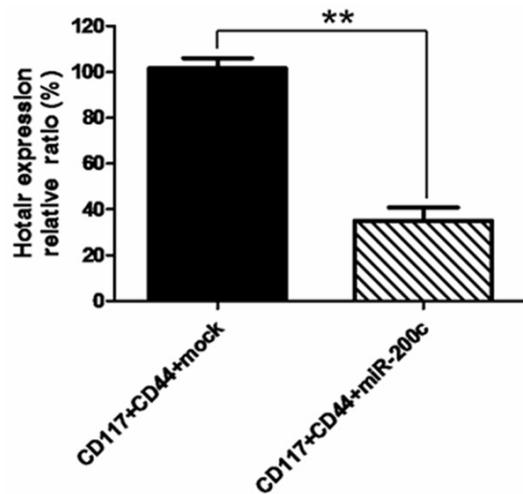
Figure 5. miR-200c regulates HOTAIR expression in SKOV3 ovarian tissues in nude mice. HOTAIR expression in the tumor tissues from the nude mice injected with the lentivirus-miR-200c transducted or lentivirus-mock transducted SKOV3 CD117$^+$CD44$^+$CSCs. **P<0.01.

patients with advanced ovarian cancer, however, a common problem faced by the EOC patients is the generated resistance to paclitaxel and cisplatin, which results in the 5-year survival rate in patients with stage III and IV EOC less than 40% [27, 28]. One of mainly reasons is that paclitaxel/cisplatin-based therapies are able to eliminate the bulk of differentiated cancer cells, but are unable to eliminate CSCs. Therefore, CSCs are an attractive population of cells to target therapeutically [13]. We based on the accumulating evidence that noncoding RNAs especially miRNAs and lncRNAs represent a significant advance towards a better understanding of the mechanisms that govern cancer cellular growth, and wanted to understand the possibility and the mechanisms of mir-200c inhibition of lncRNA HOTAIR in decreasing the chemotherapetic resistence of EOC CSCs.

Our current study has indicated that miR-200c is a HOTAIR-suppressive miRNA in SKOV3 CD117$^+$CD44$^+$CSCs, and that the inhibition function is miR-200c dependent. This is because the miR-200c was found to bind HOTAIR in a sequence-specific manner, which resulted in down-regulation of the HOTAIR expression. Down-regulation of lncRNA by miRNA has only been recently observed. The suppression of HOTAIR expression by miRNA overexpression

correlated with alteration of HOTAIR function, including proliferation, invasion, metastasis, and chemoresistance [9, 21]. Our findings indicatd the miR-200c overexpression, which targets inhibition of HOTAIR, demonstrated that the decrease of the chemoresistance of SKOV3 CD117$^+$CD44$^+$CSCs to paclitaxel/cisplatin treatment in vitro, and that suppression of expression of TGF-β1 and Bmi-1 in vivo (tumor tissues). In addition, SKOV3 CD117$^+$CD44$^+$CSCs transducted with lentivirus-miR-200c significantly inhibited its metastasis in lung tissues from the SKOV3 bearing nude mice (this mice not shown here, seen in reference 3). Our finding is essentially consistent with the previous findings by others [8, 9, 29] and by us [5, 25].

It is known that Bmi-1 is an oncogene that causes neoplastic proliferation [20], and that. TGF-β1, an EMT promoter facror, has been shown to increase stem-like properties in cancer cells [3]. Both of Bmi-1 and TGF-β1 have been found to be a target for miR-200c [18, 19]. In this study, the expression of Bmi-1 and TGF-β1 inversely correlated with the miR-200c expression in the tumor tissue samples at mRNA expression level (**Figure 2**). MiR-200c overexpression in SKOV3 CD44$^+$CD117$^+$CSCs decreased the cellular metastasis potential (**Figure 3**). From these consistent data, we guess that miR-200c overexpression specifically inhibited the zinc-finger E-box binding homeobox 1 expression in SKOV3 CD117$^+$CD44$^+$ CSCs, and reduced the expression of Bmi-1 and TGF-β1, which may inhibit the EMT, decrease the chemoresistance to paclitaxel and cisplatin as well as reduce the metastasis ability of SKOV3 CD117$^+$CD44$^+$CSCs by down-regulation of HOTAIR expression [5, Wang et al., 2014].

MiR-200c and HOTAIR are well studied among miRNA and lncRNA, and have been shown to have an important functions in normal and cancer cells [21, 24], but miR-200c and HOTAIR interaction has never been studied in ovarian carcinoma cells. However, we understand that more studies are fully warranted to find out the mechanisms of how interaction between the miR-200c and the HOTAIR in SKOV3 CD117$^+$ CD44$^+$CSCs.

In conclusion, our findings presented in this study demonstrated the miR-200c targets and decreases HOTAIR expression in EOC SKOV3

CD117$^+$CD44$^+$CSCs, and reduces cellular drug resistant to paclitaxel and cisplatin. These results suggest that interaction between the miR-200c and the HOTAIR may play a critical role to increase EOC SKOV3 CD117$^+$CD44$^+$CSC sensitivity to clinical therapies.

Acknowledgements

Disclosure of conflict of interest

None.

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