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Original article

HOTAIR may regulate proliferation, apoptosis, migration and invasion of MCF-7 cells through regulating the P53/Akt/JNK signaling pathway

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ABSTRACT

Breast cancer is a common malignancy, and it is the second leading cause of cancer-related death among women worldwide. The pathogenesis of breast cancer is poorly understood, leading to unsatisfactory efficacy of current anti-PC therapies. The aim of this study is to investigate the role of LncRNA HOTAIR in proliferation, apoptosis, migration and invasion of human breast cancer cell line MCF-7. MCF-7 cells were cultured and transfected with HOTAIR siRNA, and the proliferation rate of cells was determined using MTT and colony-forming assay; moreover, the apoptosis as well as cell cycles were determined using annexin V/propidium iodide staining methods and analyzed using flow cytometery; furthermore, cell scratch and transwell assays have been performed to examine the migration and invasion of MCF-7 cells; Next, cells were collected, and RT-qPCR as well as western blotting assay were performed to examine the expression of P53, MDM2, AKT, JNK, MMP-2 and MMP-9. We discovered that knockdown of HOTAIR induced significant decrease in proliferation and increase in apoptosis of MCF-7 cells, and the cell cycles of HOTAIR siRNA transfected cells have been arrested at G1 phase ($p < 0.01$); moreover, knockdown of HOTAIR lead to marked decrease in the migration and invasion ability of MCF-7 cells; finally, knockdown of HOTAIR induced significant decrease in the expression of P53/Akt/JNK ($p < 0.01$), and significant increase in the expression of P53 in MCF-7 cells ($p < 0.01$). In conclusion, our results proved that HOTAIR may regulate proliferation, apoptosis, migration and invasion of MCF-7 cells through regulating the P53/Akt/JNK signaling pathway.

1. Introduction

Breast Cancer(BC) is a common malignancy, and it is the second leading cause of cancer-related death among women worldwide [1]. Based on data from World Cancer Research Fund, Globally 0.45 million patients die from breast cancer annually, which solely constitutes 13.7% of female cancer deaths, and U.S.A, China and India are the top 3 countries with the highest prevalence of breast cancer [2]. In recent years, great efforts have been made to improve the efficacy of methods for the diagnosis and treatment of BC, however, the pathogenesis of BC remains poorly understood, and the efficacy of current anti-BC medications remain unsatisfactory, leading to the poor prognosis of BC. Thus, to further explore the pathogenesis of BC and identify novel diagnostic and therapeutic targets is in great demand.

In recent years, the investigations of long non-coding RNAs (lncRNAs) have become an increased area of focus. LncRNAs are endogenous RNAs with the length >200 nt, and unlike mRNAs, lncRNAs lack open reading frame (ORF), indicating that they did not have the potential for coding proteins [3]. LncRNAs used to be considered as "genetic junks", however, in some recent studies, the roles in many cellular and molecular activities(e.g. cell proliferation, differentiation, apoptosis) have been reported. In the field of cancer studies, dysregulation of lncRNAs is related to prognosis, metastasis, and recurrence in different cancer types, and the overexpression of some onco-lncRNAs (e.g. HOTAIR, MALAT1, BCYRN1, etc.) has been proved to promote the proliferation and invasion of cancer cells [4–9].

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Hox transcript antisense intergenic RNA (HOTAIR), a 2.2-kb-long transcript localized to the boundaries of the HOXC gene cluster, has an oncogenic role in several type of cancers [10–16]. Aberrant expression of HOTAIR was also observed in breast cancer tissue and cell lines [17,18], however, the roles of HOTAIR in the pathogenesis of breast cancer still requires further investigation. In the present work, we will focus on the role of HOTAIR in the proliferation, metastasis and invasion of breast cancer cell line MCF-7. We hypothesized that HOTAIR is an onco-lncrna in breast cancer, and HOTAIR can promote the malignant behaviors of the cancer cells through inhibiting the expression of the tumor suppressor p53.

2. Materials and methods

2.1. Cell culture

Human breast cancer cell line MCF-7 was purchased from cellcook(Guangzhou, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) that supplied with 10% fetal bovine serum(FBS) and penicillin/streptomycin(Gibco, Thermo Fisher Scientific, Waltham, MA, USA)at 37 °C and 5% CO2.

2.2. Cell transfection and RNA interference

To knockdown HOTAIR in MCF-7, cells were transfected with HOTAIR siRNA or empty vector (purchased from GenePharma, Shanghai, China)using Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) for 72 h according to the manufacturer's instructions. The HOTAIR siRNA sequences were: 5′-AAUUCUUAAAUUGGGCUGG-3′.

2.3. Cell proliferation assay

The proliferation rate of MCF-7 cells were determined by MTT assay (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacture's protocol 72 h after transfection.

2.4. Colony-forming assay

Colony formation assay has been performed to determine the effect of HOTAIR siRNA on the proliferation of MCF-7 cells. Briefly, the untransfected cells, HOTAIR siRNA transfected cells, and empty vector transfected cells were cultured for 7 days, and the colonies were stained using 0.1% crystal violet. Then the number of the colonies was calculated.

2.5. Cell apoptosis and cell cycle analysis

Cells was stained with the Annexin V/propidium iodide apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, USA) 72 h after transfection, and the apoptosis rate and cell cycle were analyzed using BD FACSVerse flow cytometer(BD Biosciences, Franklin Lakes, NJ, USA).

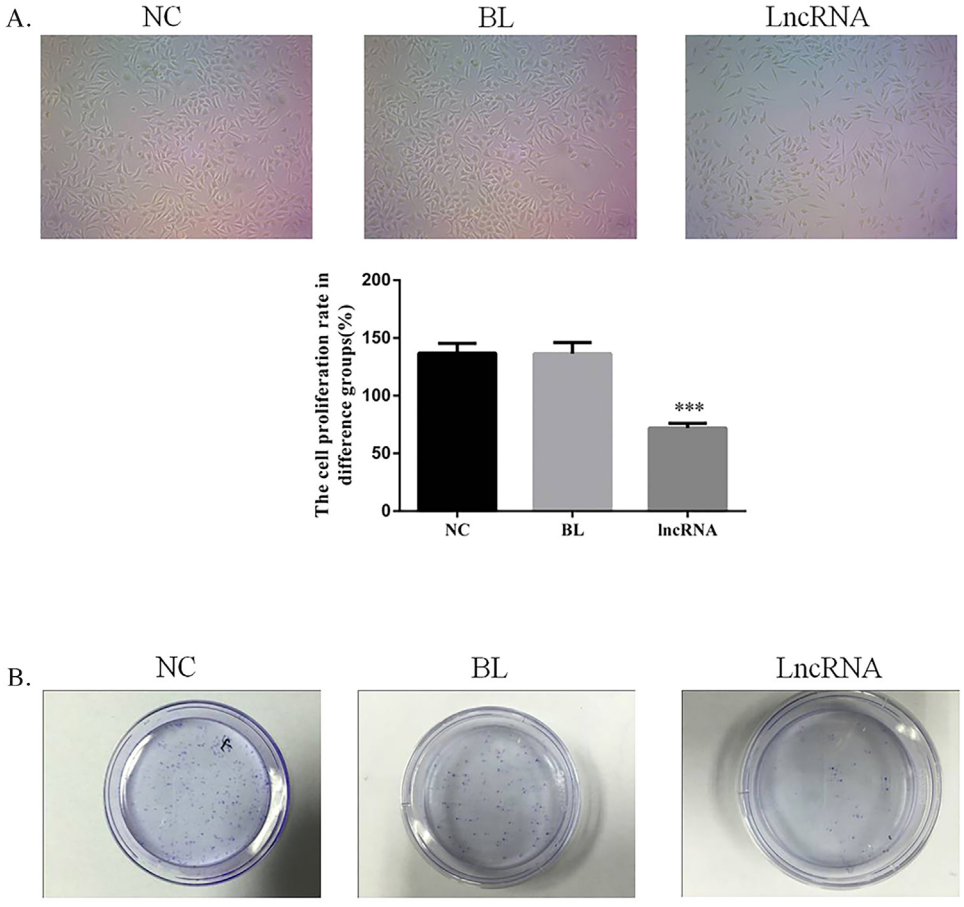

Fig. 1. Knockdown of HOTAIR inhibits proliferation of MCF-7 cells in vitro. (A) MTT assay. (B) Colony-forming assay. NC: un-transfected cells; BL: empty vector transfected cells; LncRNA: HOTAIR siRNA transfected cells. *** $p < 0.01$.