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Original article

LncRNA MEG3 has anti-activity effects of cervical cancer

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ABSTRACT

Aim: The aim of this study was to explain the lncRNA MEG3 had anti-cancer effects to suppress cervical carcinoma biological activity.
Methods: The Hela cell were divided into three groups (NC group,BL group and lncRNA group). The cells of lncRNA or BL groups were transfered with lncRNA MEG3 or blank carrier. Evaluating the cell proliferation rate of difference groups by MTT assay; measuring the cell apoptosis and cell cycle of difference groups' cell by flow-cytometry; the cell invasion activity of difference groups were measured by transwell assay, the cell migration ability of difference groups were evaluated by wound healing testing. Measuring the relative gene expressions (PI3K, AKT, MMP-2, MMP-9, Bcl-2, Bax and P21) and protein expressions (PI3K, AKT, p-AKT, MMP-2, MMP-9, Bcl-2, Bax and P21) by RT-PCR or WB assay.
Results: Compared with NC group, The cell proliferation rate of lncRNA group was significantly reduced ($P < 0.05$) and the cell apoptosis and G1 phase were significantly increased ($P < 0.05$, *respectively*). The invasion cell of lncRNA MEG3 group were significantly difference compared with NC group ($P < 0.05$), and the wound healing rate of lncRNA MEG3 group was significantly shorter than NC group ($P < 0.05$). The PI3K, AKT, MMP-2, MMP-9 and Bcl-2 gene expression of lncRNA group were significanlty down-regulation compared with NC group ($P < 0.05$,*respectively*), and Bax and P21 gene expression of lncRNA group were significantly up-regulation compared with NC group ($P < 0.05$,*respectively*) by RT-PCR testing. The PI3K, AKT, p-AKT, MMP-2, MMP-9 and Bcl-2 protein expression of lncRNA group were significanlty down-regulation compared with NC group ($P < 0.05$,*respectively*), and Bax and P21 protein expression of lncRNA group were significantly up-regulation compared with NC group ($P < 0.05$,*respectively*) by WB assay.
Conclusion: The lncRNA MEG3 had effects to supress cervical cancer by regulation PI3K/AKT/Bcl-2/Bax/P21 and PI3K/AKT/MMP-2/9 signaling pathway.

1. Introduction

Cervical cancer has been high incidence and a serious threat to women's health in young women. Cervical cancer, as a second major malignant tumor in women, still could't early diagnosis and poor prognosis. The current studies found that human papillomavirus (HPV) infection was detected in 99.8% cervical cancer patients [1,2], However, isolated HPV infection is insufficient to cause cervical cancer, the development of tumors is a multistep and multi-step process involving the activation of proto oncogenes or (and) inactivation of oncogenes. In this process, gene mutation and abnormal transcription play important roles in the cell itself must be changed can eventually cause cervical cancer, so, study on the pathogenesis of cervical cancer is particularly important [3].

Long non-coding RNAs (lncRNAs) are thought to be a "noise" of genomic transcription and a by-product of RNA polymerase II transcription, transcript length is less than 200nt and has no biological function [4]. However, in recent years' studies, the results were shown lncRNAs were involved in many important regulatory processes such as X chromosome silencing, genomic imprinting and chromatin modification, transcriptional activation, transcriptional interference and nuclear transport [5,6], and were closely related to the tumor occurrence and development, the lncRNA was difference expressions in colon cancer [7], breast cancer [8] and liver cancer [9]. At present, the related lncRNA found in cervical cancer including BC200 [10], Malat [11], MEG3 [12],

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XLOG-010588 [13] and HOTAIR [14], nevertheless, it hasn't been clearly that the mechanism of most of these lncRNAs. In this study, we investigated the effects of lncRNA MEG3 to biological activity of cervical cancer cell line Hela and its related mechanisms by over-expressing lincRNA MEG3.

2. Materials and methods

2.1. Materials

Hela cell (ATCC, USA); lncRNA (Kingsy biotech, China); MTT kit (Invitrogen, USA); Culture medium and fetal bovine serum (Hyclone, USA); Pancreatin, opti-MEM (Gibco, USA); Lipofectamine 2000, TRIzol, PureLink RNA Mini Kit, Dnase I (Invitrogen, USA); Matrigel (BD, USA); 6/24/96 well tissue culture plate (Nune, USA); Primer synthesis (Kingsy biotech, China).

2.2. Methods

2.2.1. Cell culture and transfection

Hela cells were inoculated into 6 hole plate, cultured with DMEM-F12 medium contained 10% FBS, and cultured in incubator with 37 °C and 5% CO_2;the Hela cell were divided into three groups: Control group, NC group and lncRNA group. The cell density was about 40% on the bottom of the culture bottle, The lncRNA group were transfected by lipofectamine 2000 and 200 pmol MEG3 lncRNA(F:5′-GGGCTTCGGAATGAGCATGCTACTG-3′; R:5′-ACATTC-GAGGTCCCCTTCCCACGTAGGCATC-3′), NC group were transfected by lipofectamine 2000 and 200 pmol negative lncRNA (F: 5′-UUCUCCGAACGUGUCACGUTT-3′; R: 5′-ACGUGACACGUUCG-GAGAATT-3′), Control group were treated with normal. After transfection 6 h, relaocing culture medium.

2.2.2. MTT assay

The Hela cell were digested by pancreatin, regulating the cell number to 1×10^6 cells per hole by culture medium. After incubating the cell in incubator with 5%CO_2, 37 °C overnight. After incubation with 72 h, 20 μL MTT (5 g/L) were added in every well, and the cells were cultured in the CO_2 incubator for 4 h. After that, giving up the culture fluid in the hole, adding 150 μL DMSO in every well, Room temperature oscillation, enzyme labeling instrument 490 nm wavelength measurement absorbance value, recording analysis of experimental data.

2.2.3. Cell apoptosis assay

Hela cells after digestion and counting were inoculated in 6 well plates, there were 5×10^5 cells in every well, After cell adherent growth overnight, discard cell culture medium, digesting the cell by 0.25% trypsin and collecting cells. Suspending cell by PBS, Centrifugal by 2000r/min for 5 min; discard supernatant, PBS washed cells once; Suspension the cells by 500 μL Annexin V Binding Buffer, and adding 5 μL Annexin V-FITC and 5 μL Propidium Iodide in the cell suspension, Gently mixed, room temperature, light incubation 10 min, flow cytometry analysis.

2.2.4. Cell cycle assay

Cells of difference groups with respectively treatment were collected after each culture for 24 h, washed with pre cooled PBS for about 2 times, suspended in the 75% ethanol solution, and the cells were fixed overnight; After PBS washing, adding PI (10 μg/mL) and PBS contained 0.1% RNaseA, Cell cycle was measured by flow cytometry at room temperature and 10 min.

2.2.5. Cell invasion assay

300 μL serum free medium were added in indoor addition, static set for 30 min, remove the medium, matrix gel and serum-free medium by the 1: 5 ratio dilution, Adding 100 μL dilution to each cell, static glue solidification were at the temperature of 37 °C for 30 min; After cells digesting for 12 h, the cells were collected by centrifugation at 1000g/ min, Based on the cells in serum-free medium, according to each cell 3×10^5 cells into cells, the lower 10% FBS medium containing 600 μL, At the temperature of 37 °C, 5% CO_2 under the condition of 48 h culture, remove the chamber, discard the culture medium in the hole, washed 2 times after fixed with 90% ethanol for 30 min PBS, the removal of 90% ethanol, washed 2 times with PBS, Stained with 0.1% crystal violet min of 30,

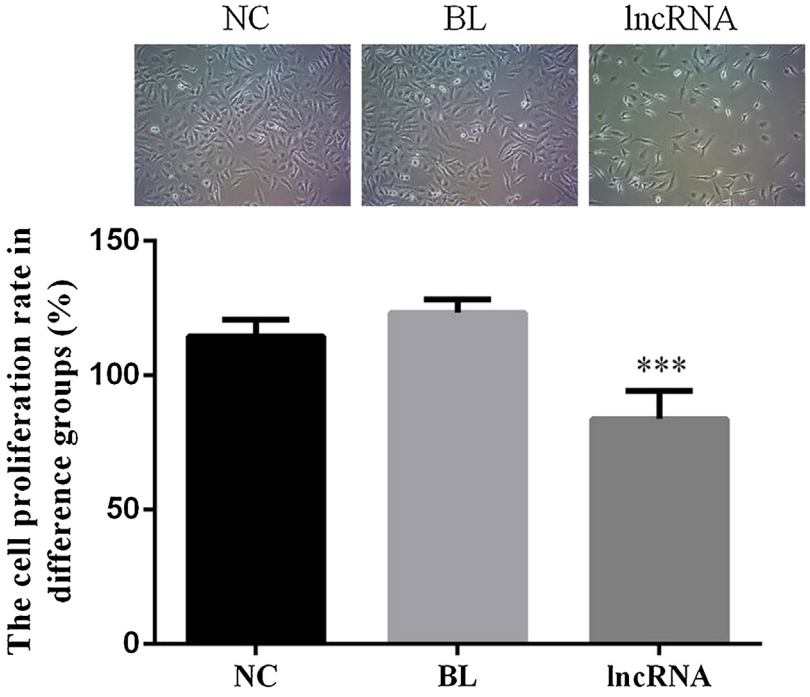

Fig. 1. The cell proliferation of difference group ***: $P < 0.05$.