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Effects of HMGA2 on malignant degree, invasion, metastasis, proliferation and cellular morphology of ovarian cancer cells

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ABSTRACT

Objective: To analyze effects of high mobility group AT-hook 2 (HMGA2) on malignant degree, invasion, metastasis, proliferation and cellular morphology of ovarian cancer cells. **Methods:** Three methods were applied to observe the effect on HMGA2 expression in ovarian cancer cells and ovarian epithelial cells. **Results:** After the application of siRNA-HMGA2, number of T29A2- cell clones was decreased, there was significant difference compared with the negative control Block-iT. After application of let-7c, number of T29A2+ cell clones was decreased significantly, however, after the application of Anti-let-7, the number of clones restored, and there was no significant difference compared with the negative control group. After interference, the number of T29A2- cells which passed through Matrigel polycarbonate membrane were significantly lower than the negative control group. After the treatment of siRNA-HMGA2, let-7c and sh-HMGA2 respectively, growth and proliferation of T29A2-, T29A2+ and SKOV3 were slower, and the phenomenon was most obvious in SKOV3. Stable interference of HMGA2 induced mesenchymal-epithelial changes in the morphology of SKOV3-sh-HMGA2. **Conclusions:** HMGA2 can promote malignant transformation of ovarian cancer cells, enhance cell invasion and metastasis, and promote cell growth and proliferation of ovarian cancer cells, which can cause ovarian cancer to progress rapidly and affect the quality of life.

1. Introduction

Ovarian cancer is a malignant cancer which locates on ovary, most of ovarian cancers are primary and the morbidity is the third among female reproductive system malignant cancers which is just next to cervix cancer and urine uterine cancer[1,2]. The malignant degree of ovarian cancer is high, and affect life quality of patients significantly. Morbidity and progress mechanism is the key in clinical treatment. High mobility group AT-hook 2 (HMGA2) is involved in various biological processes such as the formation of the embryo and the development of malignant cancer[3]. We aimed to analyze its effects on the malignant degree, invasion, metastasis, proliferation and cellular morphology of ovarian cancer cells.

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2. Materials and methods

2.1. Materials

2.1.1. Cell lines

Stable high H-RAS protein expression cell line T29H mutated from ovarian cancer epithelial cell lines T29, T80 and T29 was selected. Ovarian cell lines SKOV3, HEY, OVCAR-3, Caov-3 and cells packed by Phonenix virus were selected as experimental cell lines in our research[4-6]. All above cell lines were purchased from American standard center culture collection (American Type Culture Collection).

2.1.2. Instruments and equipments

MTS kits were provided by Promega Corporation for cell proliferation and preserved at minus 20℃. 96-well plates

were purchased from American Scientific Corporation.

2.1.3. Cell culture media

The cell culture medias including dulbecco's modified eagle medium, heat inactivated fetal bovine serum, M199 culture media, epithelial growth factor, complete medium, pancreatin-EDTA(0.05% and 0.25%), cell freezing medium, and Dulbecco's phosphate buffered saline were obtained from Sigma-Aldrich Corporation, the preparation methods were referred to related references[7-11].

2.2. Interference methods

2.2.1. Production of transient transfection reagents

20 nmol siRNA-HMGA2 (20 μM) powder was obtained and dissolved in 1ml sterilized distilled water to be divided and preserved in refrigerator. let-7c and anti-let-7c: 5 nmol above powder was obtained and dissolved in sterilized distilled water without RNA enzyme to be divided and preserved in refrigerator. 20 μM control small RNA was obtained and divided to be preserved. Lipofectamine 2000 was directly preserved in refrigerator at constant 4℃[12,13].

2.2.2. Transfection methods

The cells were cultured and transfected when the confluence was up to 40%-50%[14-19]. to T29A2- and T80A2- cells was interfered by HMGA2-siRNA; T29A2+ cells was interfered by let-7c. Transfect HMGA2 expressed ovarian cancer SKOV3-sh-HMGA2 cell line was established by Lentivirus package. SKOV3-pGIPZ was used as control cell line. Fluorescent staining was used to verify the interference result after 48 h.

2.3. Observation indicators

Soft AGAR cloning formation experiment, Matrigel invasion assay, growth curve method[20,21] were applied to observe malignant degree of cells, ability of invasion and metastasis, and proliferation ability, then the morphological change among cells was compared.

2.4. Statistical analysis

All date in our study were analyzed by SPSS13.0. Enumeration data was analyzed by *Chi*-square test and measurement data was analyzed by *t* test. The test level was set as α =0.05. The difference was considered as statistically significant when $P<0.05$.

3. Results

3.1. Interference effects

Florescent staining method showed there was satisfactory interference effects in different cells, as shown in Figure 1.

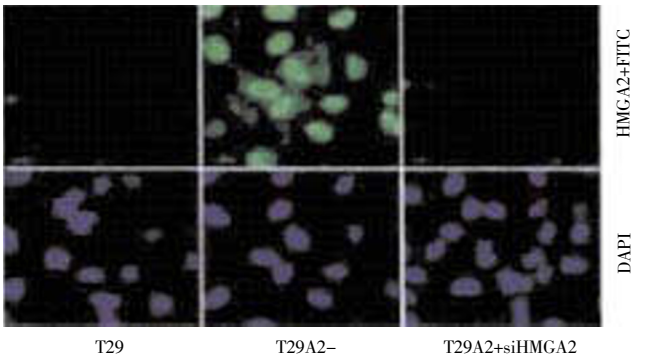

Figure 1. Interference effects of siRNA-HMGA2 tested by florescent staining.

3.2. Malignant degree

After the application of siRNA-HMGA2, number of T29A2- cell clones was significantly decreased compared with the negative control Block-iT (t=13.509, 12.214, respectively, all $P<0.05$). After the application of let-7c, number of T29A2+ cell clones was decreased significantly, however, after the application of anti-let-7, the number of clones restored, and there was no significant difference compared with the negative group, as shown in Figure 2.

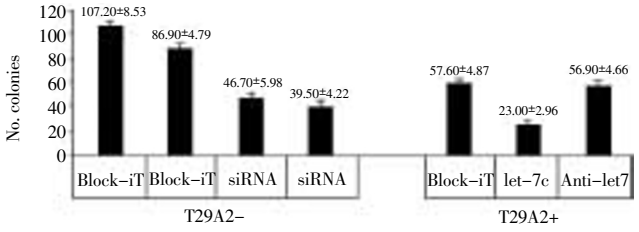

Figure 2. Results of cell line malignant degree by soft AGAR cloning formation experiment.

3.3. Ability of invasion and metastasis

After interference, the number of T29A2- cells which passed through Matrigel polycarbonate membrane was significantly lower than the negative control group, as shown in Figure 3.

Table 1
Comparison of 96 h cell growth condition(mean±sd).

Type of cell	T29A2-	T29A2+	SKOV3
HMGA2 interfered	49 885.6±1 827.5	73 855.5±2 629.6	231 829.7±15 338.6
Negative control	69 405.2±4 345.0	99 705.6±3 058.4	402 981.0±6 598.5
t	37.423	22.950	79.365
P	<0.05	<0.05	<0.05

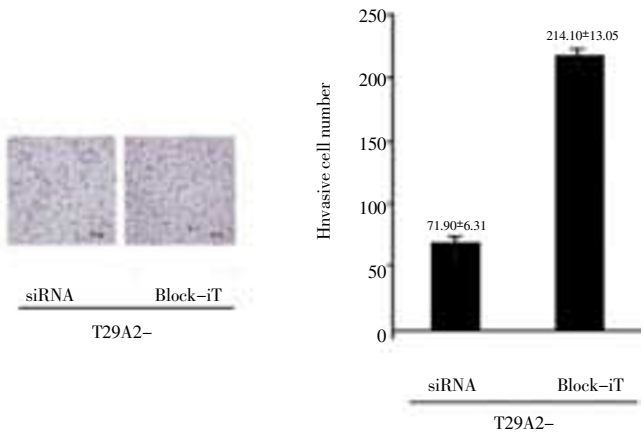

Figure 3. Matrigel invasion assay result of cell line invasion and metastasis ability.

3.4. Ability of proliferation

After the treatment of siRNA-HMGA2, let-7c and sh-HMGA2 respectively, proliferation of T29A2-, T29A2+ and SKOV3 was slower, and the phenomenon was most significant in SKOV3, as shown in Table 3 and Fig 4.

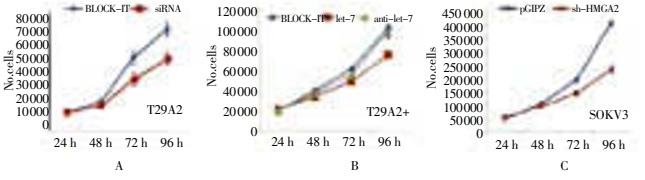

Figure 4. Growth curve analysis of cell line proliferation ability.

3.5. Cell morphology

Stable interference of HMGA2 induced mesenchymal-epithelial changes in morphology of SKOV3-sh-HMGA2 (Figure 5).

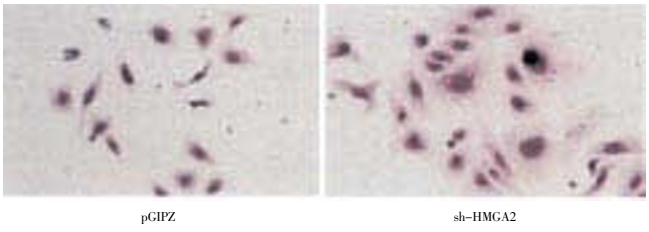

Figure 5. Cell morphology results.

4. Discussion

Ovarian cancer is divided in to type Ⅰ and type Ⅱ, malignant degree of type Ⅰ including mucous carcinoma, clear cell carcinoma and endometrioid carcinoma is low, however, type Ⅱ has high morbidity and progresses rapidly, 5-year survival rate of which is less than 30% and there has been no effective therapy[22,23]. Thus, to analyze the biological behavior, mechanism of occurrence and development has become critical in guiding clinical treatment.

HMGA2 is a gene located at 12q13-15 which can code and construct transcriptional factor proteins. Dahiya *et al*[24-27] found that HMGA2 showed significant effects on the nerve system development, pituitary adenoma development and DNA injury impairment in mouse, and was verified as a critical oncoprotein of many malignant cancers.

To explore the effects of HMGA2 on malignant degree, invasion, metastasis, proliferation and cellular morphology of ovarian cancer cells, we applied siRNA, let-7c and anti-let-7c to interfere the expression of HMGA2. After the application of siRNA-HMGA2, number of T29A2- cell clones decreased, there was significant difference compared with the negative control Block-iT; after the application of Let-7c, number of T29A2+ cell clones decreased significantly, however, after the application of anti-let-7, the number of clones restored, indicating that after interference of HMGA2 expression, the malignant degree of ovarian cancer significantly decreased; Matrigel invasion assay showed the ability of ovarian cell invasion and metastasis significantly decreased after interference of HMGA2 expression; after the treatment of siRNA-HMGA2, let-7c and sh-HMGA2 respectively, proliferation of T29A2-,T29A2+ and SKOV3 was slower, and the phenomenon was most obvious in SKOV3, indicating the cell growth and proliferation were affected significantly by interference of HMGA2, and the HMGA2 stably interfered cells were affected most[28]. In observation of cellular morphology, we found SKOV3-sh-HMGA2 cells had mesenchymal-epithelial change after HMGA2 interference, and epithelial-mesenchymal transition (EMT) plays important roles in development of various biological processes such as early embryo, invasion and metastasis of cancer and fibrosis of chronic inflammation[29,30]. We consider that the reason why HMGA2 affects the malignant degree, invasion, metastasis, proliferation ability of ovarian cancer is that it is involved in the formation of EMT to promote the malignant transformation, enhance the ability of invasion and metastasis and promote the proliferation of cells. Thus ovarian cancer progresses rapidly and affects the survival quality.

In conclusion, we can apply the HMGA2 expression level interference as the targeted therapy to inhibit the progress of cancer and improve the prognosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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