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Association Examined of Viscoelastic Properties with the Invasion of Ovarian Cancer Cells by Atomic Force Microscopy

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Running title: Viscoelastic Properties and Cell Invasion

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Abstract

Cellular mechanical properties could serve as a prominent indicator for disease progression and early cancer diagnosis. This study utilized atomic force microscopy (AFM) to measure the viscoelastic properties and then examined their association with the invasion of ovarian cancer at living single cell level. The results demonstrated the elasticity and viscosity of ovarian cancer cell OVCAR-3 and HO-8910 significantly decreased than those of HOSEpiC, the ovarian cancer control cell. Further examination found the dramatic increase of migration/invasion and the obvious decease of microfilament density in OVCAR-3 and HO-8910 cells compared with those of HOSEpiC cells. And there was a significant relationship between viscoelastic and biological properties among these cells. In addition, the elasticity was significantly increased in OVCAR-3 and HO-8910 cells after the treatment of anticancer compound echinomycin (Ech), while no obvious change was found in HOSEpiC cells after Ech treatment. Interestingly, Ech seemed no effects on the viscosity of these cells. Furthermore, Ech significantly inhibited the migration/invasion and significantly increased the microfilament density in OVCAR-3 and HO-8910 cells compared with those of HOSEpiC cells, which was significantly related with the elasticity among these cells. Notably, an increase of elasticity and a decrease of invasion were found in OVCAR-3 and HO-8910 cells with Ech treatment. Together, this study clearly demonstrated the association of viscoelastic properties with the invasion of ovarian cancer cells and shed a light on the biomechanical changes for early diagnosis of tumor transformation and progression at single cell level.

Keywords: atomic force microscopy; invasion; migration; ovarian cancer cells; viscoelasticity;

1 Introduction

Ovarian cancer is a lethal gynecological malignancy among females with low survival rates due to the fact that the disease is generally diagnosed during the late stages [1, 2]. Discriminating more tumorigenic cancer cells from less tumorigenic types contributes to the determination of disease severity and personalized treatment [3]. Notably, a close relationship between the progression of cancer and the change of cell mechanical properties has been discovered in the last decades [4, 5]. Mechanical properties used to grade the tumorigenic and metastatic potential of cells are strongly associated with cell transformation, migration and invasion. Therefore, the cells diseased could be detected biomechanically.

At present, a variety of research technologies, such as optical tweezers, micropipette aspiration, magnetic twisting cytometry and atomic force microscopy (AFM), have been developed to characterize the mechanical properties of biological samples [6-9]. Among these, AFM is widely used because of the convenience of sample preparation and the ability to examine accurately mechanical properties. Studies using AFM have revealed viscoelastic properties become the novel indicators which can be used to differentiate the cancerous cells from their healthier [10].

As we all known that cell migration and invasion are the two key processes leading to the spread of cancer cells from primary tumors to distant organs during the tumor metastasis [11, 12], which are largely related to cytoskeleton structure [13, 14]. The rearrangement of microfilament skeleton is of great necessity to the cell motility, whereas contributing largely to the elasticity changes of these cells, when cytoskeleton structure changes from more organized to disordered with the transformation from benign to malignant [15]. But the association of viscoelastic properties with the invasion of ovarian cancer cells is not well understood.

1 In addition, chemotherapy are approved as the most effective treatment for
2 advanced-stage ovarian cancer [16-20], while few researches focus on the variability
3 in mechanical properties related with cancer invasion after an anticancer drug
4 treatment [21, 22]. Echinomycin served as a potential therapeutic agent are found to
5 induce cell apoptosis, which is typically used in the treatment of epithelial cancers,
6 including ovary, breast and prostate [23-26]. Inhibitory mechanism of cancer invasion
7 and metastasis by chemotherapy can be beneficial for both biomechanical research
8 and clinical applications [27]. Therefore, the present study examined the elasticity and
9 viscosity through AFM, the migration, invasion and microfilament density through cell
10 experiment, and the relationship between them, which will shed a light on the
11 mechanism of viscoelastic property-related invasion and metastatic behaviors in
12 ovarian cancer cells.
13
14 **2. Results and discussion**
15 **2.1 Viscoelastic properties of ovarian cancer cells**
16 The viscoelastic properties of cells include elasticity and viscosity, which are
17 prominent biomechanical properties of cells. This study, the cell viscoelasticity was
18 derived from the force indentation curves obtained with AFM on three different
19 ovarian cancer cells shown in Figure 1.
20 The results of cell elasticity showed a concentrated and narrow distribution in
21 OVCAR-3 and HO-8910 cells (Figure 1a-ii,iii) and a dispersive and broad distribution
22 in HOSEpiC cells (Figure 1a-i). Further analysis of elasticity values demonstrated the
23 average values of OVCAR-3 and HO-8910 cells were 1195.72±122.94 Pa and
24 996.27.0±52.56 Pa respectively (Figure 1a-iv), which were significantly lower than
25 that of HOSEpiC cells (2160.94±167.77 Pa, Figure 1a-iv), further indicating the lower

1 elasticity of ovarian cancer cells transformed from benign to malignant. It is consistent
2 with the previous reports that the stiffness of normal cells is higher than that of breast
3 cancer cells [30]. Apparently, the present results further suggested the more
4 tumorigenic and aggressive potential of OVCAR-3 and HO-8910 cells than that of
5 HOSEpiC cells and the relationship with their mechanical properties. Therefore, the
6 elasticity could be considered as an effective indicator to differentiate the state of
7 tumor development.

8 Viscosity is another important characteristic used to reflect the viscoelastic
9 response of cells to force stimulation [3], and to represent the main energy dissipated
10 during the force indentation process [31]. The results of cell viscosity showed the
11 average values of OVCAR-3 (11.38 ± 0.72 Pa-s) and HO-8910 (10.70 ± 0.66 Pa-s)
12 were significantly lower than that of HOSEpiC (30.00 ± 0.66 Pa-s, Fig.1b), which is
13 consistent with the tumorigenic and aggressive characteristics of ovarian cancer cells.
14 It is noticeable that the viscous behaviors display the capabilities of motility and
15 invasion of cells [32, 33] , and could be used to differentiate the cancerous cells from
16 the healthier [34, 35]. The low viscosity of ovarian cancer cells with increased
17 capabilities of motility and aggression suggested increased tumorigenic potential is
18 associated with decreased cell viscosity, which has been demonstrated in previous
19 studies [3].

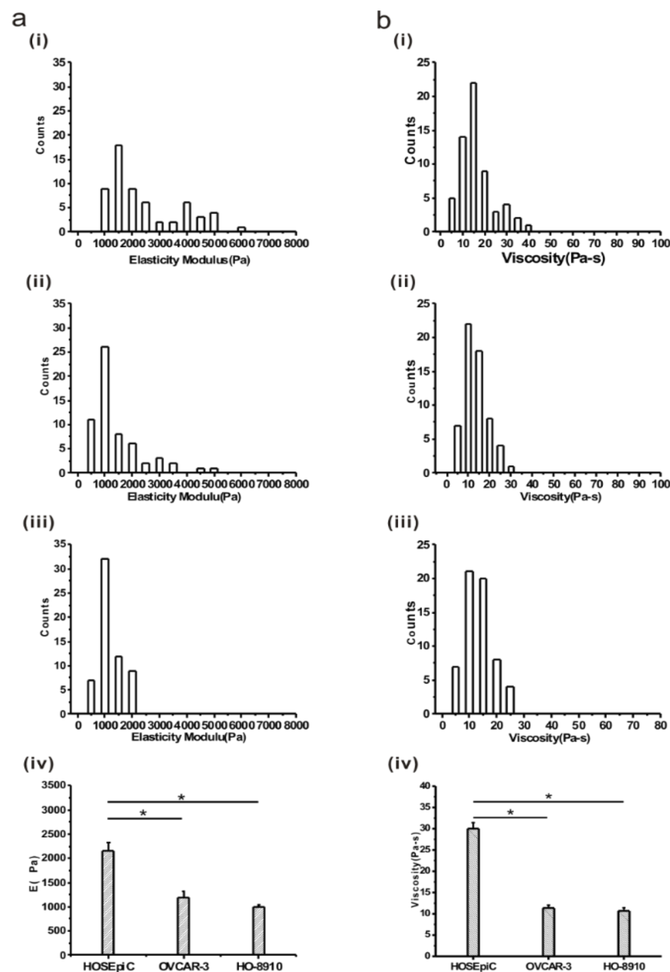


Figure 1 Histograms of viscoelastic properties for ovarian cancer cells.

Cell elasticity and viscosity were examined by atomic force microscopy. a-(i): elastic histograms of HOSEpiC cells, a-(ii): elastic histograms of OVCAR-3 cells, a-(iii): elastic histograms of HO-8910cells, a-(iv): average elastic values of ovarian cancer cells. b-(i): viscosity histograms of HOSEpiC cells, b-(ii): viscosity histograms of OVCAR-3 cells, a-(iii): viscosity histograms of HO-8910 cells, a-(iv) average viscosity values of ovarian cancer cells. The data were present as Mean ± SE, and the asterisk indicated $p<0.05$, n=60.

2.2 Tumorigenic properties of ovarian cancer cells

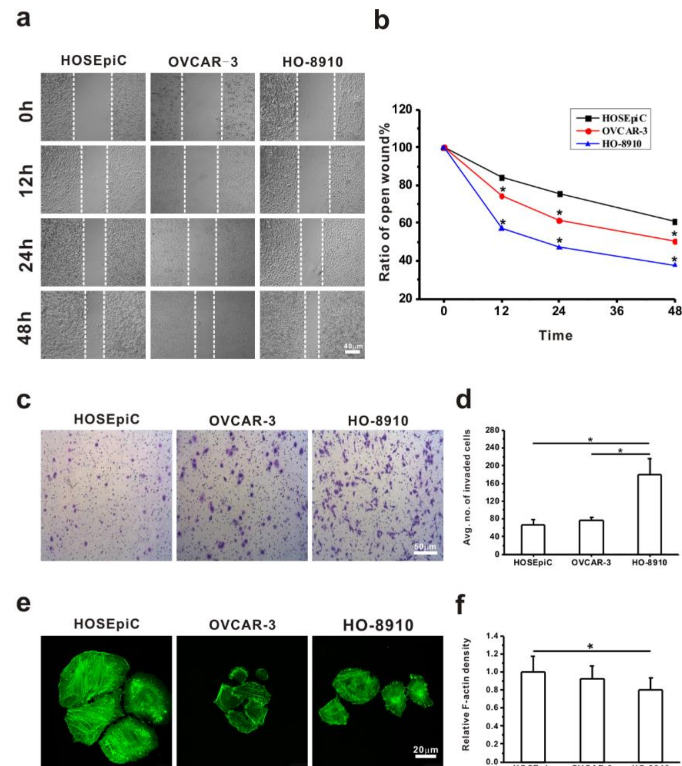
In order to clarify the relationship of viscoelastic with tumorigenic properties of ovarian cancer cells, the present study further examined the migration and invasion changes of these cells besides the microfilament density of F-actin cytoskeleton, and then analyzed the correlation between viscoelastic and tumorigenic properties of these cells.

The migration of ovarian cancer cells is critical for their tumorigenic properties and examined by the experiment of cell migration assay [36]. The present results showed the average healing rate of OVCAR-3 and HO-8910 was significantly greater than that of HOSEpiC (Fig.2a and b), which is consistent with the changes of viscoelastic results by AFM, indicating the relationship between migratory potential and viscoelastic properties of ovarian cancer cells.

The invasion of ovarian cancer cells is another tumorigenic property and examined by the experiment of cell invasion assay [37]. The present results showed the average numbers of invasive cells in the group of OVCAR-3 and HO-8910 cells were more than that in the group of HOSEpiC cells (Fig.2c) and HO-8910 cells had more invasion potential than HOSEpiC and OVCAR-3 cells (Fig.2d), which is also consistent with the changes of viscoelastic results by AFM, indicating the relationship between invasion potential and viscoelastic properties of ovarian cancer cells.

Furthermore, the microfilament density was examined by the imaging of cytoskeleton F-actin and ActinGreen (KeyGEN BioTECH) was used to investigate the distribution of actin cytoskeleton among these cells. The present results showed the actin filaments of HOSEpiC cells were distributed more extensively and organized better than that those of OVCAR-3 and HO-8910 cells (Fig.2e) and the density of actin filaments in HOSEpiC cells was also higher (Fig.2f), demonstrating the microfilament density was related to the viscoelsticity of these cells based on AFM,

1 which was also consistent with previous reports of cancer cells with the lower
2 density/distribution of F-actin filament and the larger elasticity compared with normal
3 cells [38-40].



4

5 **Figure 2 Analysis of tumorigenic properties for ovarian cancer cells.**

6 The migration and invasion of ovarian cancer cells were analyzed and the
7 microfilament density was examined by the imaging of cytoskeleton F-actin. a: The
8 cells of HO-8910, OVCAR-3 and HOSEpiC were cultured for 0h, 12h, 24h and 48h
9 and the healing of cell scratches was observed. Bar = 40 μm. b: The migration of
10 ovarian cancer cells was calculated and the asterisk indicated $p<0.05$. c: The invasion
11 of ovarian cancer cells was examined. Bar = 50 μm. d: The invasion of ovarian cancer
12 cells was analyzed and the asterisk indicated $p<0.05$. e: The microfilament density
13 was examined by the imaging of cytoskeleton F-actin. Bar = 20 μm. f: The
14 microfilament density was analyzed and the asterisk indicated $p<0.05$.

15

2.3 Correlation of viscoelastic and tumorigenic properties among ovarian cancer cells

In the present study, the correlation was analyzed (Table 1) and further confirmed the elastic properties was significantly related to the migration and invasion of ovarian cancer cells (Table 1), which may be caused by the difference of the density/distribution of F-actin filament (Table 1). The initial AFM results have identified that OVCAR-3 and HO-8910 cells were much softer and more deformable than HOSEpiC cells, and the relationship was explored in present study between viscoelastic properties and tumorigenic potential among these cells. Rebelo's group also reported the similar results that the reduction of viscoelasticity was related with the increase of the migratory potential for cancer cells, providing a new understanding of the mechanism in cancer development [41].

The process of invasion and metastasis is based on the movement and deformation of cancer cells [41], and this process is related to the viscoelasticity of cells [31]. In the present AFM experiments, the elasticity and viscosity of OVCAR-3 and HO-8910 cells were lower than that of HOSEpiC cells, which further emphasized the role of viscoelastic properties in cell invasion, which is consistent with previous report that the elasticity and viscosity decreased with increasing tumorigenic potential of cells, indicating that the malignant transformation of cells is associated with the decrease of mechanical properties [42]. Therefore, analyzing the viscoelastic characteristics of cells contributes to further understanding the ability to deform and metastasize of cells, thus further predicting the development of cancer [42].

Given the cytoskeleton plays a key role in the maintenance of cell morphology, mobility deformation and related information transmission, which become an important factor determining the mechanical properties of cells [42]. Some

researchers have shown that alterations in cytoskeletal structure or cell functional defects are associated with the ability of tumor cells to proliferate [43]. Actin filaments are important components of the cytoskeleton, and the distribution of actin filaments on the cell membrane could affect the elasticity of the cells. Therefore, the present results indicated the cytoskeletal organization is related to the viscoelastic properties of ovarian cancer cells.

Table 1 Correlation analysis of elasticity and migration, invasion and F-actin density of three ovarian cells.

Elasticity		HOSEpiC	OVCAR-3	HO-8910
Migration	HOSEpiC	-0.732		
	OVCAR-3		-0.991	
	HO-8910			-0.9528
Invasion	HOSEpiC	-0.788		
	OVCAR-3		-0.987	
	HO-8910			-0.990
F-actin density	HOSEpiC	0.963		
	OVCAR-3		0.932	
	HO-8910			0.941

2.4 Effects of anticancer compound echinomycin on the viscoelastic properties of ovarian cancer cells

For further identify the relationship of viscoelastic with tumorigenic properties among ovarian cancer cells, the present study utilized anticancer compound echinomycin (Ech) to treat these cells with 0 μM, 0.25μM and 0.5 μM for 3 h,

respectively, and then examine the changes of cell viscoelasticity after Ech treatment (Fig.3a and b).

The results of cell elasticity showed the average elasticity of HO-8910 cells exposed to 0.5 μM (2944.02 ± 238.88 Pa) Ech for 3h was higher than that with 0 μM (control, 1187.30 ± 54.27 Pa) and 0.25 μM (2377.22 ± 235.98 Pa) Ech (Fig.3a). The average elasticity of OVCAR-3 cells treated with 0.25 μM Ech increased approximately 57% compared with the control (Fig.3a), while no obvious changes of the average elasticity was found among HOSEpiC cells treated with 0 μM, 0.25 μM or 0.5 μM Ech (Fig.3a). These findings demonstrated ovarian cancer cells OVCAR-3 and HO-8910 with low elasticity and Ech-treated cells with increased elasticity, implying the effect of Ech was related with the increased elasticity of ovarian cancer cells, which is consistent with previous report that Ech induced the alterations on biomechanical properties of cancer cells [44]. The effects of drug on the biomechanical properties of cancer cells showed that the cell elasticity was increased with the increase of the drug treatment concentration, and the viscoelastic properties of cancer cells could be changed by antineoplastic drugs [45]. These results were discussed in relation to the underlying mechanical mechanism of action for Ech in ovarian cancer cells [46].

The results of cell viscosity showed the average viscosity of HOSEpiC, OVCAR-3 and HO-8910 cells treated with 0.25 μM Ech for 3 h was 24.11 ±1.81 Pa-s, 13.89 ± 1.03 Pa-s and 16.73 ± 0.89 Pa-s, respectively (Fig.3b). While treated with 0.5 μM Ech for 3 h, they were changed to 26.6 ± 2.36 Pa-s, 18.72 ±1.46 Pa-s and 16.6 ± 1.16 Pa-s, respectively (Fig.3b). Interestingly, no obvious changes of average viscosity of HOSEpiC, OVCAR-3 and HO-8910 cells were found after Ech treatment (Fig.3b). Therefore, the detailed mechanisms related to the viscosity of ovarian

cancer cells still need to be further investigated. Owing to the Ech treatment, the alterations in cell biomechanical properties showed good agreement with the drug-mediated activation in both cells [47]. The results suggested that a study of the changes on biomechanical properties in cancer cells using AFM could provide an important implication for evaluating the anticancer activity of a drug [48].

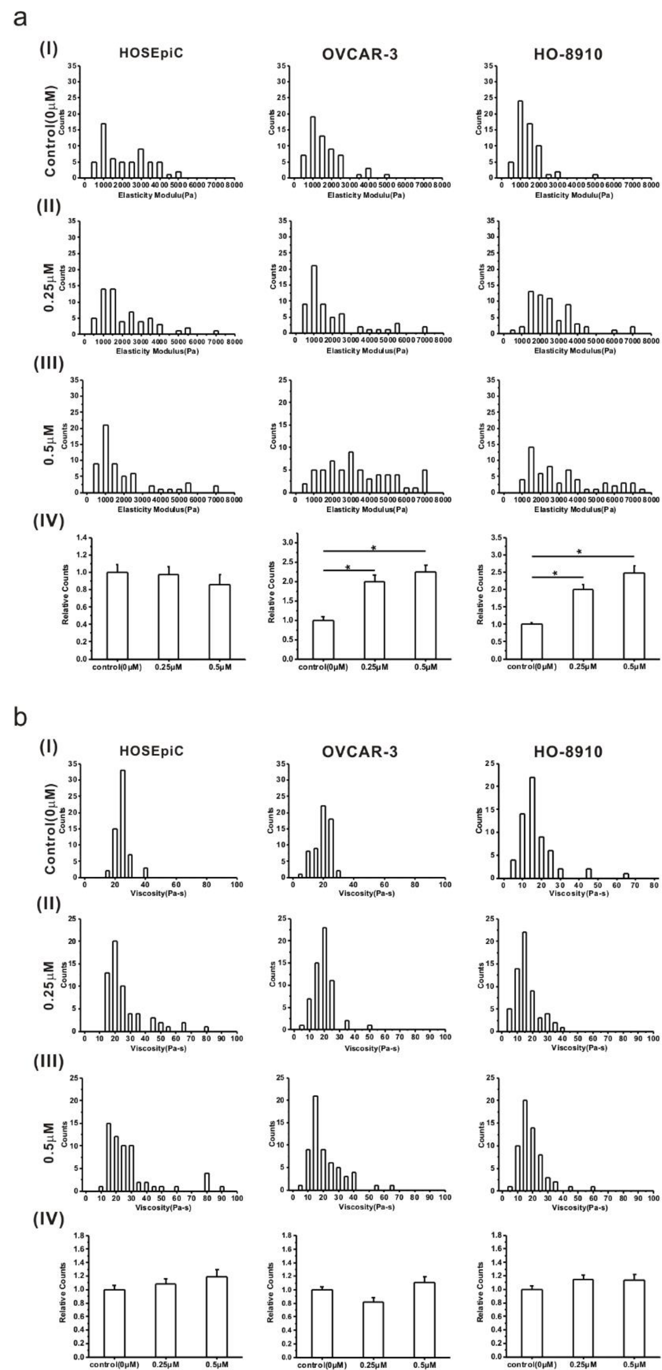


Figure 3 Histograms of viscoelastic properties for three ovarian cells.

The cells of HO-8910, OVCAR-3 and HOSEpiC were cultured and treated with 0 μM (control), 0.25 μM or 0.5 μM echinomycin. a: the elasticity of ovarian cancer cells in each group. b: the viscosity of ovarian cancer cells in each group. The data were present as Mean ± SE, and the asterisk indicated $p<0.05$, n=60.

2.5 Effects of anticancer compound echinomycin on the tumorigenic properties of ovarian cancer cells

In order to clarify the effect of Ech on the changes of tumorigenic properties, the present study examined the migration (Fig.4) and invasion (Fig.5) changes of these cells after Ech treatment as designed besides the microfilament density of F-actin cytoskeleton (Fig.6).

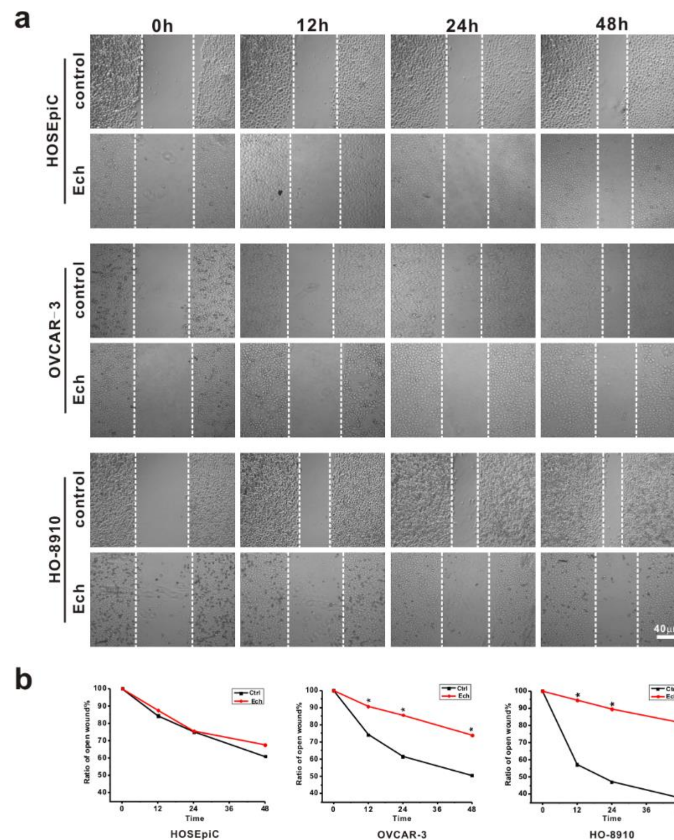
During cell migration assay, the cells were scratched and treated with 0.25 μM echinomycin for 3h, and then cell migrations were recorded respectively at different time points for exploring whether Ech affected the migratory potential of these cells. The present results showed the migratory potential was inhibited after exposure to 0.25 μM Ech compared with the control group without Ech in OVCAR-3 and HO-8910 cells, not in HOSEpiC cells (Fig.4a and b). These findings were consistent with previous report that chemotherapy drugs could efficiently suppress the migration of cancer cells [49, 50].

During cell invasion assay, the invasive cell number of HO-8910 and OVCAR-3 cells treated with 0.25 μM Ech for 3h was much lower than that of the control (Fig.5a and b), while no obvious changes were found in HOSEpiC cells (Fig.5a and b), implying a greater impact of Ech on the invasive capacity of HO-8910 and OVCAR-3 than that of HOSEpiC cells, which was consistent with the changes of AFM results. Together, these results further illustrated the mechanical properties of cells are

related to their invasive potential. Cancer cells need deformation to conduct a series of biological behaviors, such as migration and invasion [51]. Paula's group showed that the main problem in the treatment of cancers may be their invasive behaviours [52]. The present investigations indicated that chemotherapy drugs could alter the cellular mechanical properties of malignant tumors to attenuate cell proliferation, migration and invasion [53]. Lian et al have indicated that a drug inhibited cellular invasion through affecting biomechanical properties of cancer cells [54]. Ech could affect invasion activity of the cell lines in this report, and could present a new treatment regimen for malignant tumors [55].

For understanding the molecular mechanism regulating the relationship between the viscoelastic and tumorigenic properties among ovarian cancer cells, the microfilament density of F-actin cytoskeleton was examined by fluorescence imaging in these cells treated with 0.25 μM Ech for 0h, 3h and 6h, respectively (Fig.6a and b). The results showed the F-actin cytoskeleton of HOSEpiC cells with remarkable regular networks and had no obvious change after 0.25 μM Ech treatment (Fig.6a and b), while the microfilament of F-actin cytoskeleton disturbed and the density increased in HO-8910 and OVCAR-3 cells treated by 0.25 μM Ech for 3h and 6h, respectively (Fig.6a and b). These results demonstrated the changes of F-actin cytoskeleton may contribute to the changes of tumorigenic properties in ovarian cancer cells treated with Ech, further implying the relationship of viscoelastic with tumorigenic properties related with the difference of F-actin cytoskeleton among ovarian cancer cells. The variations of tumorigenic properties and tumor progression are accompanied by remodeling of the cytoskeleton. Earlier predictions have supported that the viscoelastic properties related with highly invasive cancer cells could be associated with difference of F-actin cytoskeleton [56, 29]. Each of these

1 tumorigenic transformation processes is regulated by the dynamic biomechanical
2 behaviors of a diverse varieties of F-actin cytoskeleton within the examined ovarian
3 cancer cells, and the possible underlying mechanical properties were subsequently
4 characterized [57, 58].



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6 **Figure 4 The migration analysis of ovarian cancer cells treated with**
7 **echinomycin.** The cells of HO-8910, OVCAR-3 and HOSEpiC were cultured and
8 treated with 0.25 μM echinomycin. a: the healing of cell scratches was observed from
9 0h to 48h. Bar = 40 μm. b: the migration of ovarian cancer cells was analyzed. The
10 data were present as Mean ± SE, and the asterisk indicated $p<0.05$.

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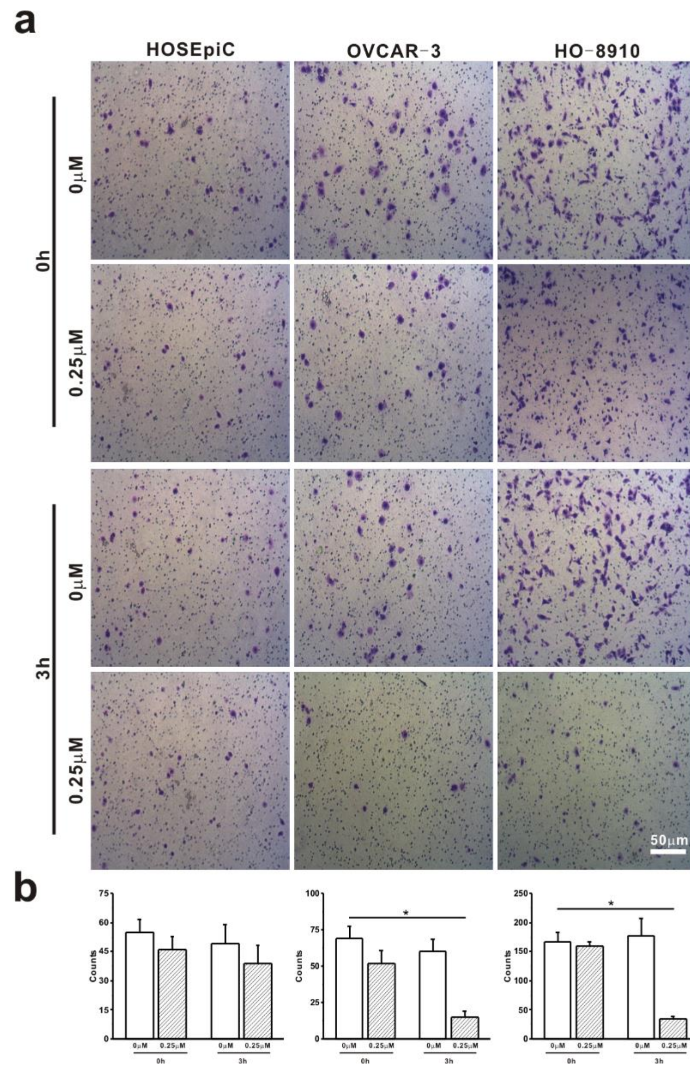

2 **Figure 5 The invasion analysis of ovarian cancer cells treated with echinomycin.**

3 The cells of HO-8910, OVCAR-3 and HOSEpiC were cultured and treated with 0.25

4 μM echinomycin for 3h. a: the invasion of ovarian cancer cells were examined. Bar =

5 50 μm. b: the migration of ovarian cancer cells was analyzed. The data were present

6 as Mean ± SE, and the asterisk indicated $p<0.05$.

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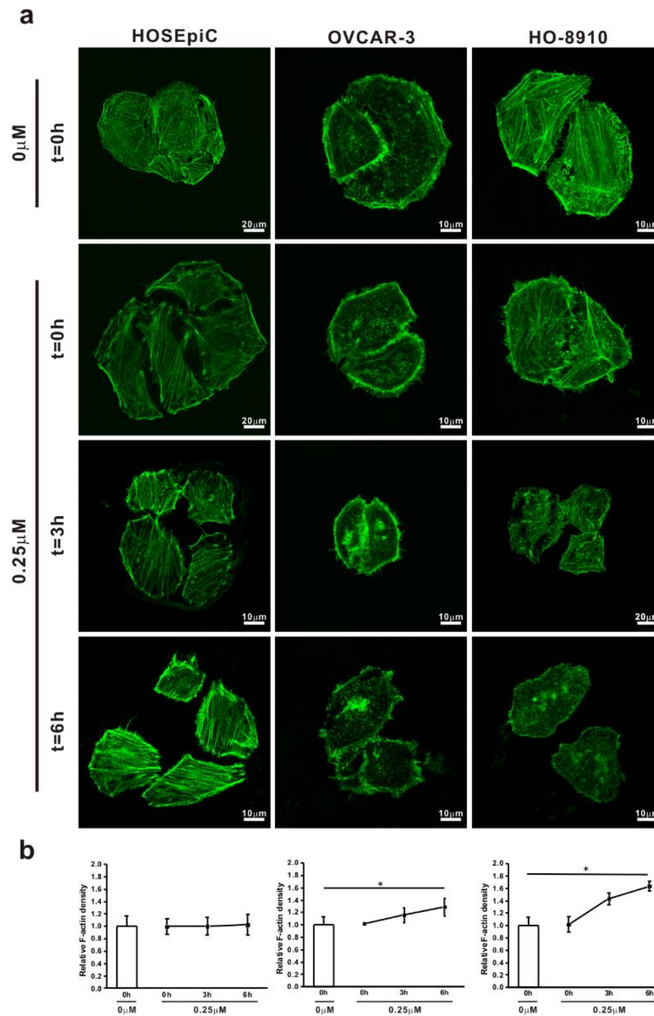

2 **Figure 6 Analysis of the microfilament density through cytoskeleton F-actin**

3 **imaging.** The cells of HO-8910, OVCAR-3 and HOSEpiC were cultured and treated

4 with 0.25 μM echinomycin for 3h. a: the microfilament density was examined by the

5 imaging of cytoskeleton F-actin. Bar = 20 μm. b: The microfilament density was

6 analyzed. The data were present as Mean ± SE, and the asterisk indicated $p<0.05$.

7

8 **2.6 Correlation of viscoelastic and tumorigenic properties among ovarian**

9 **cancer cells treated with Ech**

10 Notably, the correlation was analyzed (Table 2) and further confirmed the elastic

11 properties was significantly related to the migration and invasion of ovarian cancer

12 cells (Table 2), which may be caused by the changes of the density/distribution of

F-actin filament in these cells after Ech treatment (Table 2). Furthermore, the changes of average elasticity and cell invasion were analyzed after exposure to Ech (Fig.7) and the results showed no obvious changes of average elasticity (Fig.7a) and cell invasion (Fig.7c) in HOSEpiC cells, while a significant increase in OVCAR-3 and HO-8910 cells after Ech treatment (Fig.7a and c). Interestingly, the cell invasion of OVCAR-3 and HO-8910 cells were obviously increased compared with that of HOSEpiC cells (Fig.7d), which was also consistent with the changes of average elasticity among these cells (Fig.7b), further indicating the relationship of elastic and tumorigenic properties among ovarian cancer cells, but the detailed mechanism need further to be investigated in the future.

Table 2. Correlation analysis of elasticity and migration, invasion and F-actin density after exposure to echinomycin.

Ech- elasticity		HOSEpiC	OVCAR-3	HO-8910
Migration	HOSEpiC	-0.936		
	OVCAR-3		-0.872	
	HO-8910			-0.910
Invasion	HOSEpiC	-0.915		
	OVCAR-3		-0.983	
	HO-8910			-0.869
F-actin density	HOSEpiC	0.833		
	OVCAR-3		0.926	
	HO-8910			0.845

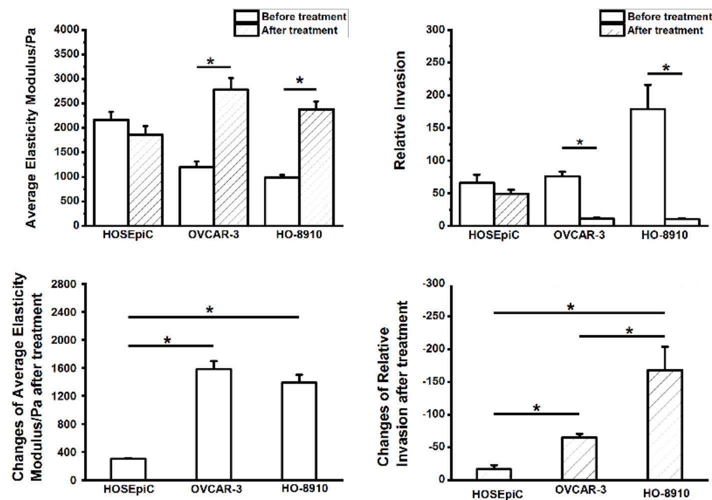

Figure 7 Analysis of average elasticity and invasion changes in ovarian cancer cells treated with echinomycin. a: the average elasticity of ovarian cancer cells with and without echinomycin treatment. b: the changes of the average elasticity after echinomycin treatment. c: the relative invasion of ovarian cancer cells with and without echinomycin treatment. d: the changes of the relative invasion of ovarian cancer cells after echinomycin treatment. The data were present as Mean ± SE, and the asterisk indicated $p<0.05$.

3 Conclusions

To our knowledge, the present study firstly demonstrated the association examined of viscoelastic properties with the invasion of ovarian cancer cells by atomic force microscopy. The present results not only found the more malignant degree, the lower viscoelasticity found in ovarian cancer cells, but also found the migratory and invasive potential increased with the decreased cell viscoelasticity. Furthermore, the results of anticancer compound echinomycin treatment experiment suggested the association of cell elasticity with the invasion of ovarian cancer cells may be caused by the differences of F- actin cytoskeleton. Together, the present study not only provide a new method to investigate the invasive mechanisms of

ovarian cancer cells, but also promise AFM as an effective analytical approach during very early diagnosis of cancers at living single cell level.

4 Materials and methods

4.1 Cell culture

Three ovarian cell lines, HOSEpiC (human ovarian epithelial cell line, BeNa Culture Collection, Beijing, China), OVCAR-3 (human cancerous ovarian cell line, BeNa Culture Collection, Beijing, China) and HO-8910 (human cancerous ovarian cell line, BeNa Culture Collection, Beijing, China), were purchased and used. HOSEpiC and HO-8910 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution, while OVCAR-3 cells were grown in RPMI 1640 with 20% FBS, 0.01mg/ml bovine insulin and 1% penicillin-streptomycin solution. These cells incubated and cultured at 37℃ in a humidified atmosphere of 5% CO_2. For AFM experiment, the ovarian cancer cells were seeded in 35 mm culture dish with a density of 1×10^4/ml for 24 hours. Different concentrations of echinomycin were added into the culture dish for 3h stimulation, respectively. After the treatments, cells were then washed twice with PBS and immediately used for AFM measurements in 2 ml medium.

4.2 Viscoelastic measurement

The viscoelasticity of cells were measured via AFM (Nano Wizard III, JPK, Berlin, Germany) equipped with an inverted optical microscope (Leica, Germany) in the experiments.The viscoelastic properties were investigated under Force-Spectroscopy working mode. The cultured dish was placed in the Petri Dish Heater (JPK instrument, Berlin Germany) maintaining at 37°C during the AFM indentations. Force-distance

curve-based AFM were obtained to calculate the optical photodiode deflection sensitivity and the cantilever spring constant was verified by the thermal noise method before experiments. MLCT cantilevers (Bruker, USA) made of silicon nitride with approximate spring constant values of 0.01N/m were employed in all AFM experiments. Scanning station was selected in the areas surrounding the nuclei of cells (3 μm×3 μm) in medium at room temperature. The indentation force was 1nN with a constant velocity of 5 μm/s. To reduce the experimental error, the measurements of samples were applied under the same conditions. All data were analyzed using the JPK data processing software [28]. The elasticity modulus was acquired based on Hertz model, and then the viscosity was calculated [29].

4.3 Assay of cell migration

The cells were seeded in the 6-well plates at a density of 8×10^6/well/ml culture medium, and were cultured until the confluences reached approximately 95%. The cell confluent monolayer was wounded using a sterilized 10 μL pipette tip, and then washed three times with PBS to remove dislodged cells. The culture medium was also changed to the serum-free medium. The area of wound closure was taken pictures with an inverted microscope. Then the samples were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for later analysis. Cells migrated into the surface area of wound closure and the average distance of migrating cells were monitored by collecting digitized images at the designated time-point.

4.4 Assay of cell invasion

The invasion of ovarian cancer cells was analyzed by Cell Culture Insert (Corning, 8.0 μm pore size) coated with PET membrane according to the

manufacturer's instructions. A total of $1×10^4$ cells suspended in 500 μl serum-free medium was loaded into the upper chambers, and the bottom chamber was filled with 500 μL medium contain 10% FBS to stimulate invasion. The cells were incubated for 24 h and then the invading cells in the bottom of the chamber insert were stained with Giemsa. Numbers of invading cells were photographed and calculated at five randomly selected sites. Each assay was conducted at least three times.

4.5 Confocal Imaging of microfilament skeleton

The cytoskeletal organization in ovarian cancer cells was investigated by confocal imaging. The cells were seeded into 35 mm cover glass bottom culture dishes (Nest) at a density of $5×10^4$ cells per milliliter, and cultured in the 37℃ incubator for 2 days prior to staining. Then, culture medium was removed and 1 ml PBS was added to each culture dish. After washed three times with PBS, the samples with or without echinomycin treatments were fixed by 4% paraformaldehyde (PFA) for 15 min, and 0.1% Triton-X-100 was used for permeabilization. After that, the cells were stained with ActinGreen (KeyGEN BioTECH). A laser scanning confocal microscopy (SP8, Leica) was used to image the cytoskeletal organization by F-actin. The fluorescence imaging was captured at 488 nm excitation wavelength. Moreover, the images were processed with the software Image J.

4.6 Statistical analysis

The data were reported as mean ± error (SE). Independent-samples t test was used to analyze the difference between two groups. Statistical analysis was conducted using SPSS software. The value of $P < 0.05$ was considered statistically significant.

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MC, JZ, ZW and HY conceived and designed the experiments. MC and JZ performed the experiments. ZZ carried out a part of the biological experiments. WR and YW analyzed the data, MC was a major contributor in writing the manuscript. ZW, SX and HY reviewed the manuscript. All authors read and approved the final manuscript.

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