Investigation of paclitaxel effects on morphological and micromechanical properties of human endometrial adenocarcinoma cells by AFM

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ABSTRACT

We investigated the effects of paclitaxel on cell survival, apoptosis, morphology, and biophysical properties of human endometrial adenocarcinoma cells (Ishikawa cells). The changes in the biophysical property induced by paclitaxel of the Ishikawa cells was measured by atomic force microscopy (AFM). Cell viabilities and proliferations were analyzed using the methylthiazol tetrazolium (MTT) assay. The proliferation rate decreased as the paclitaxel dose and treatment time increases. After paclitaxel treatment, apoptosis occurred after 24 h. The surface roughness of cells increased, while stiffness decreased due to morphological changes induced by treatment with paclitaxel. From these results, we could conncluded that the paclitaxel significantly damaged the Ishikawa cells not only in proliferation but also morphology and biophysical properties.

Keywords: atomic force microscopy, Ishikawa cell, paclitaxel

1 INTRODUCTION

Paclitaxel is a new antineoplastic agent that is commonly used in the treatment of human carcinomas [1-6]. The paclitaxel have been shown promising potential in the treatments of epithelial cancers, such as breast, ovary, lung, and colon [1,2]. The mechanism of action of paclitaxel has been considered to be unique as a microtubule-stabilizing agent. It binds to the β-subunit of tubulin and promotes the formation of stable microtubules in tumor cells. Resultantly, the dynamic instability of cell decreases but the microtubule rigidity increases, thereby inhibiting cell replication through disruption of normal mitotic spindle formation [1,2]. Additional activities of paclitaxel have been reported in various tumor cells. The paclitaxel induces apoptosis which is dependent on FAS-associated death domain protein through activation of caspase-10 but is independent on death receptors [3]. Also, the paclitaxel regulates the expression of apoptosis-related proteins like Bbcl-2, Bad, Bbcl-X, p21-waf, tumor necrosis factor 1 (TNF-a), and the tumor necrosis factor related apoptosis-inducing ligand (TRAIL) receptors DR4 and DR5 [4-6]. However, the exact mechanism underlying paclitaxel induced apoptosis in different cell lines and under different stimuli still remain unclear.

Recently, it was suggested that morphological changes and biomechanics of cancer cells treated by anticancer drug will help to evaluating anticancer activity of a drug [7,8]. This is because of that the antimicrotubule drug affects the shape of the cell and the mechanical properties of the cancer cell, such as roughness and stiffness, which is related to the cell functions of adherence, motility, transformation, and invasion. These changes in the morphology and mechanical property of individual cells can be detected by atomic force microscopy (AFM). Since the AFM is a very high-resolution type of scanning probe microscopy, it has been shown to be a powerful tool for imaging materials at the nanometer scale and observing the ultrastructure of the cell [9,10]. Particularly, it appropriate for measuring changes of the micromechanical properties in the cell [11,12]. This research is in the front of the stage, therefore there are only few reported studies about the morphology and mechanical property changes of cancer cell after anticancer drug treatment.

In the present study, through measuring image, stiffness, and roughness of cells using AFM, we showed that the

shape and mechanical property of cancer cells are significantly changed by treatment with paclitaxel. The changes were also dependent on the treatment conditions. The cell viability and proliferation were analyzed using methylthiazol tetrazolium (MTT) assay.

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2 MATERIAL AND METHOD

Cell Culture

Ishikawa cells were derived from a well differentiated human endometrial epithelial adenocarcinoma. The cell was grown in Minimum Essential Medium (Gibco, Auckland, New Zealand) containing 5% FBS (Sigma, USA) and antibiotics (100 U/ml 122 of penicillin and 100 μg/ml of streptomycin) and 2 mM glutamate. The cells were cultured on plastic substrata (SPL, USA) in a humidified atmosphere of 5% CO_2 at 37 ℃.

Sample fixation

The cells were fixed by immersion in 2.5% gluteraldehyde for 30 min. Cells were then rinsed with PBS buffer solution and stored at room temperature without the light.

MTT assay

Cell viability and proliferation were analyzed using methylthiazol tetrazolium (MTT) assay. This colorimetric assay was based on the ability of live cells to reduce yellow MTT reagent (Sigma, St Louis, MO, USA) to a purple formazan product. Cells were seeded in 12 well (SPL, Tissue Culture Testplate) by different times (0, 4, 12, 24, 48 h). After incubation, MTT was aspirated and 100 μl per well of DMSO (Dimethyl sulfoxide) was added to each well. Subsequently, ELISA reader read the optical densities of plates at 540 nm.

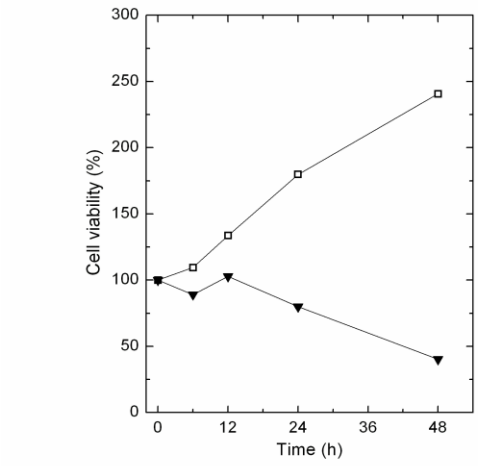

Fig. 1. Cell viabilities of Ishikawa cell. The cells were seeded (2×10^5) and treated by varying time (0 ~ 48 h). The open rectangle and filled triangle are the results of the nontreated and treated at 50 μM, respectively.

Atomic force microscopy

AFM system used in this work, which is NANOStation II (Surface Imaging Systems, Herzogenrath, Germany), consists of an AFM scanner and Zeiss optical microscope (Epiplan 500x). The images of cancer cell were measured by the reflex-coated gold cantilever in contact mode (Budget Sensor, Bulgaria). The material property and dimension of probe used in the contact mode were: resonance frequency of 13 kHz (± 4 kHz), force constant of 0.2 N/m (± 0.14 N/m), cantilever length of 450 μm (± 10 μm), cantilever width of 38 μm (± 5 μm), cantilever thickness of 2 μm (± 1μm), tip radius of 5 nm (± 1 nm), and tip height of 17 μm (± 2 μm). All images were taken in PBS buffer solution with 256 × 256 pixels and a scan speed of 0.2 line/s. The scan area depends on the size of cancer cell (between 40 × 40 μm^2 and 60 × 60 μm^2). To minimize elastic effects for obtaining high-resolution images, the samples were scanned at a low imaging force of < 0.2 nN [13]. Image processing and data analysis were performed by the SPIPTM software (Scanning Probe Image Processor version 4.1, Image Metrology, Denmark).

3 RESULTS AND DISCUSSION

Cell viability

The effect of paclitaxel on the proliferation of Ishikawa cell was investigated as a function of time. Figure 1 shows the proliferation rates of the Ishikawa at paclitaxel of 0 and 50 μM. In Ishikawa cells, it was observed the significant dependence of the proliferation rate on the paclitaxel treatment conditions. At 12 h treatments, the proliferation rates were slightly increased. After then, the rates was significantly decreased compare to the initial value.

Morphological property

The high resolution AFM images were obtained with Ishikawa cells which were fixed by 2.5% GA for 30 min for optimized imaging condition. The cells were imaged in PBS buffer solution in order to avoid size and shape distortions caused by drying. Fig. 2(a) shows the representative AFM images of the untreated Ishikawa cell which is a control sample. Figs. 2(b)-(f) show the images of the treated Ishikawa cell at different treatment times of 6, 12, 24, 36, and 48 h, respectively. The different colors in the image indicate the different height; the light and dark colors correspond to the higher and the lower topography, respectively. The untreated Ishikawa cell showed the conventional shape having distinct boundary, smooth surface, and centrally located nuclear. As shown in Fig. 2(b), there was no typical morphological change for 6 h taxol treatment. But when the Ishikawa cells were exposed to taxol for more than 12 h, obvious apoptotic morphological changes were observed. It was frequently observed aggregations, cytoplasmic vacuoles,

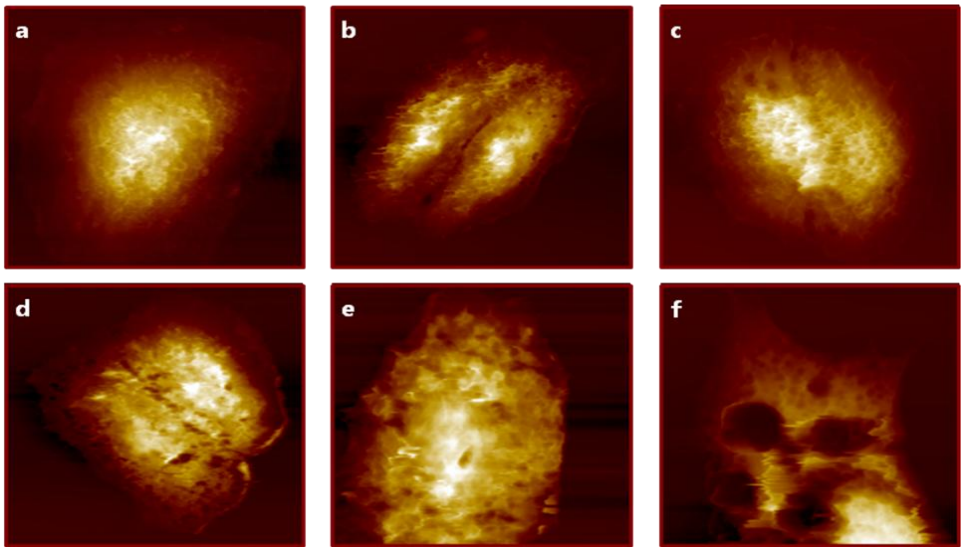

Fig. 2. AFM images of Ishikawa cell treated with paclitaxel 50 mM for 0(a) 6(b), 12(c), 24(d), 36(e), and 48(f) h. The color represents the height of surface topography: the light and dark colors correspond to the higher and the lower topography, respectively.

micronucleated cells, and floated cells. Most interesting thing is that the cell membrane was severely damaged as shown in Figs. 2(c)-(f). It was seen many holes on the cell membrane.

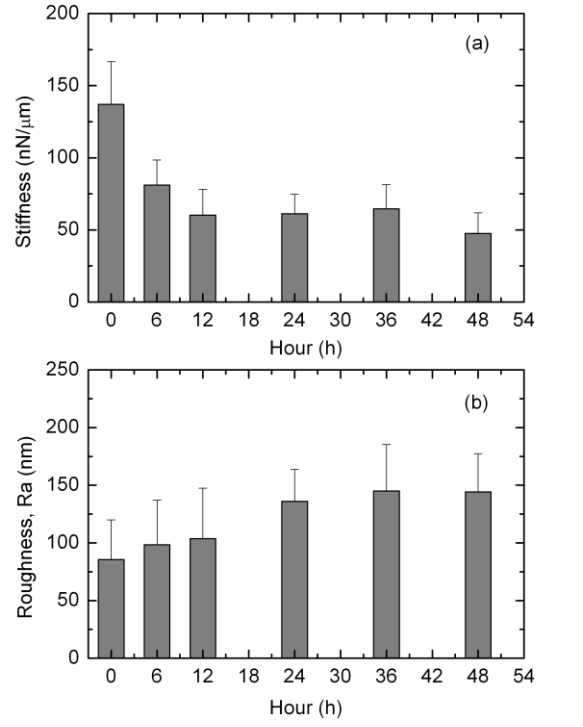

Fig. 3. The results of stiffness (a) and roughness (b) as a function of the paclitaxel treatment time.

Biophysical property

Fig. 3(a) shows the results of stiffness as a function of the paclitaxel treatment time. Interestingly, the stiffness was significantly decreased by the taxol treatment. The stiffness of nontreated cell was 137 which is largest value, but it was decreased by 60% at 6 h. The stiffness was decreased more by 44% at 12 h, but there was no significant change in the stiffness at h > 12 h. Fig.3(b) shows the result of roughness of the cell membrane. For each cell line and treatment, 3 or 4 cells were analyzed independently. The surface roughness was calculated for 20 randomly selected area of 3×3 μm^2. In all treated Ishikawa cells, the average roughness (Ra) was greater compared to the untreated cell. The value of Ra of the untreated cell was 66.4 ± 26.8 nm and it was increased to 125.9 ± 54.8 nm as the treatment time increases to 24 h. Further increasing the paclitaxel treatment increased the roughness more. From the AFM measurement, the morphological changes after the paclitaxel treatment were investigated. In Insikawa cell, typical apoptotic morphological changes were observed, and the changes became more significant as the treatment time increases.

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