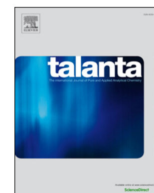
Talanta 191 (2019) 461–468

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta



Directly observing alterations of morphology and mechanical properties of living cancer cells with atomic force microscopy

Nan Wang[a,b,c,1], Miaomiao Zhang[a,d,1], Yaqing Chang[a,c], Niu Niu[a,d], Yanxue Guan[a,c], Ming Ye[e], Chen Li[a,d], Jilin Tang[a,c,d,*]

[a] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China*
[b] *School of Pharmacy, Hebei Medical University, Shijiazhuang 050017, PR China*
[c] *University of Chinese Academy of Sciences, Beijing 100049, PR China*
[d] *University of Science and Technology of China, Hefei 230026, PR China*
[e] *Bruker (Beijing) Scientific Technology Co., Ltd, Shanghai 200233, PR China*

ABSTRACT

Epithelial-mesenchymal transition (EMT) is a biological process during which cells lose their characteristic structure and biochemical properties then adopt typical features of a mesenchymal phenotype. Alterations in the morphology, structure, and mechanical properties of cells during EMT are associated with a series of pathological processes. In this work, atomic force microscopy (AFM) is used for investigating effects of TGF-β1 on morphology and mechanical properties of living bladder cancer cells (T24) during EMT for the first time. High-resolution topography and Young's modulus images of T24 living cell are obtained simultaneously. The results show that TGF-β1 is able to induce EMT, leading to the increased F-actin stress fibers and much higher Young's modulus values of T24 living cells. It reveals that the cytoskeletal-associated cell architecture is closely related to the mechanical dynamics of T24 cells during EMT. This work provides new insights into the changes of cell morphology and mechanical properties during EMT. It enables us to gain a deeper understanding of the growth, development and metastasis of the bladder cancer cell therefore it is of great significance for studying the pathological mechanism of cells at single-cell level.

1. Introduction

Epithelial-mesenchymal transition (EMT) is a biological process during which cells lose their structure and biochemical properties then adopt typical features of a mesenchymal phenotype. In the process of EMT, cell-cell and cell-matrix adhesions decrease, and the migration capacity of cell is enhanced [1]. Thus, EMT plays an important role in the process of tumor metastasis [2]. One of cytokines which can promote EMT is transforming growth factor-β1 (TGF-β1). At the late stage of tumor, TGF-β1 can promote the process of EMT, leading to alterations in cell morphology, structure and mechanical properties [3]. Alterations in morphology, structure, and mechanical properties of cells during EMT are associated with a series of pathological processes [4]. Therefore, it is important to study the morphological and mechanical properties of living cancer cells at single-cell level during EMT in physiological conditions for investigating pathological processes.

Atomic force microscopy (AFM) has the advantages on quantifying the mechanical properties of biological specimens [5,6]. Thus, AFM is widely applied to investigate the cell physical properties [7–12]. To understand the correlation between the morphology and mechanical properties in living cells, a key issue is to simultaneously obtain the high-resolution images of the cell morphology and mechanical properties. However, traditional AFM imaging method, usually referred to as force-volume mode [13,14], acquires a small array of low loading rate force-distance curves (typically 1 Hz). It suffers from poor resolution (normally a lateral resolution of a few micrometers). Thus, it is difficult to obtain high-resolution images of the mechanical properties. In addition, the cell structures that are soft and flexible can be easily displaced by forces applied during the scanning process. Therefore, obtaining high-resolution images of the cell morphology and mechanical properties simultaneously in liquid is still a challenge, and only a few works have been reported so far [15–17].

Peakforce tapping operating mode is a new quantitative AFM-based imaging technique [18]. In peakforce tapping operating mode, z piezo is driven with a sinusoidal waveform and AFM tip is oscillated in the vertical direction at a high frequency (typically 1 kHz). The maximum probe–sample interaction force of each curve is used to control vertical forces, usually as some tens of pico-Newton (Fig. 1B). Therefore, it can effectively reduce the lateral force, which is ideally suited to image soft samples with nanometer scale resolution [19–21]. Optimal image

* Corresponding author at: State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China.

E-mail address: jltang@ciac.ac.cn (J. Tang).

[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.talanta.2018.09.008
Received 27 June 2018; Received in revised form 29 August 2018; Accepted 3 September 2018
Available online 05 September 2018
0039-9140/ © 2018 Elsevier B.V. All rights reserved.

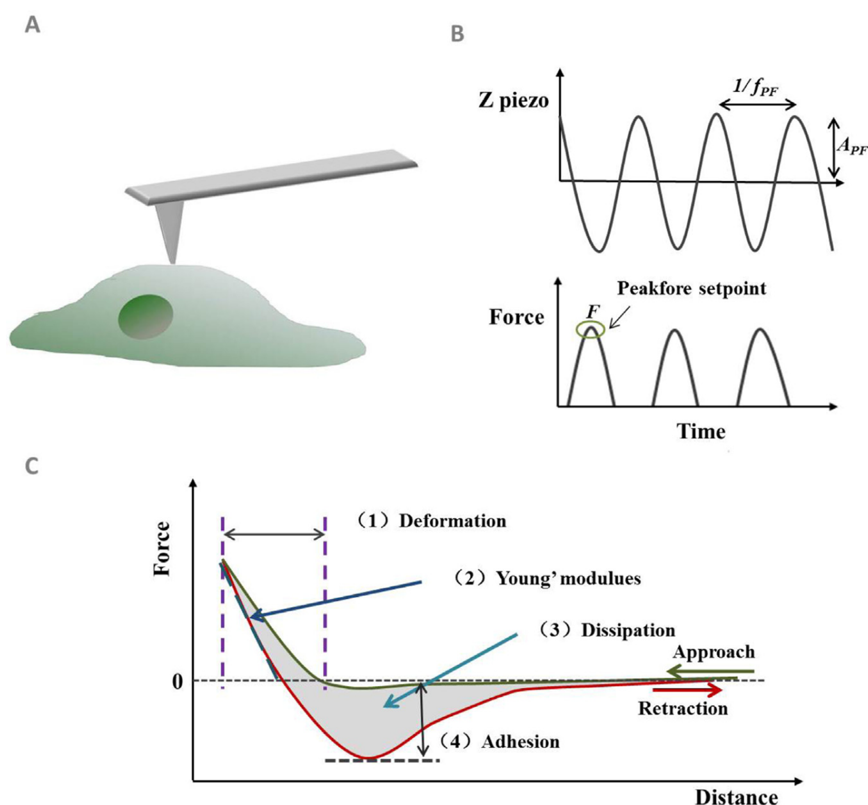


Fig. 1. Schematic representation for imaging living cells in liquid media by peakforce tapping operating mode. (A) The AFM tip is oscillated in the vertical direction at a high frequency on the surface of cell. (B) The peak values of the periodic force (peakforce setpoint) as a consequence of sinusoidal actuation on the cantilever (at frequency f_{PF} with amplitude A_{PF}) are used for feedback control. (C) Real time measurements of the force distance are provided, so that the resulting values of the mechanical properties, including deformation, Young's modulus, adhesion force, energy dispersion are displayed as an image: (1) The maximum sample deformation is calculated from the force zero point to the peakforce setpoint along the approach. (2) In order to obtain the Young's modulus image, the retract curve is fitted using the Derjaguin–Muller–Toporov (DMT) model. (3) The energy dispersion is given by the area between the approach and retraction curves integrated in one vibration period (represented by the grey area in the C) (4) Adhesion force is illustrated by the highest negative force.

acquisition and quantitative measurements of the mechanical properties, including the deformation, Young's modulus, adhesion force, energy dispersion and other information, can be obtained simultaneously by peakforce tapping operating mode (Fig. 1C). Peakforce tapping operating mode offers an unprecedented potential for label-free, imaging of topography and mechanical properties of living cells with a high resolution [22,23]. It is well suited for studying the morphology, structure and mechanical properties of living cells.

Here, peakforce tapping operating mode was used for the first time to directly observe alterations of morphology and mechanical properties of living bladder cancer cells (T24) during EMT induced by TGF-β1. High-resolution topography and Young's modulus images of T24 living cell were obtained simultaneously. The correlation between increased Young's modulus values and F-actin stress fibers observed in topography was also investigated. Moreover, the cytoskeleton arrangement was characterized by immunofluorescence confocal microscopy to investigate the effect of TGF-β1 on F-actin stress fibers.

2. Experimental section

2.1. Cell culture and treatment

T24 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS), under a humidified atmosphere of 5% CO_2 at 37 °C. After 1 day, cells were cultured in medium containing 1% FBS for 12 h. Then the cells were treatment with TGF-β1 (at a final concentration of 10 ng/ml) in medium containing 10% FBS. For control experiments, the cells were cultured in medium containing 10% FBS without TGF-β1.

2.2. AFM imaging

Before AFM experiments, the cells were washed using phosphate buffer saline (PBS) for six times. All data were acquired in RPMI-1640 medium environment at 37 °C using Bioscope Resolve AFM (Bruker, USA). To guarantee the pH value is close to the physiological conditions of cell culture, all images were acquired within 2 h. Peakforce tapping operating mode was performed by using PFQNM-LC-A-CAL AFM tips (Bruker), and the tip radius was 70 nm. We used a set point peak-force in the range of 0.2–0.25 nN, and a scan rate of 0.3 Hz with the resolution of 256 samples per line. The z-piezo position was oscillated at 1 kHz, and the amplitude of the oscillation was set at 300 nm. The spring constants of the cantilevers were determined by the thermal-noise method [24].

A total of 42 cells were measured and analyzed in this study. To obtain Young's modulus imagines, the controller software (NanoScope 9.30 Software, Bruker) fitted the retract curve with the Derjaguin–Muller–Toporov (DMT) model [25] acquiescently (Fig. 1C, Fig. 2). In this experiment Poisson's value $\nu = 0.5$ was assumed, and the force fit boundary was 30–90%. Data analysis was performed with NanoScope Analysis 1.8 (Bruker).

2.3. Immunofluorescence assays

The cells were washed with PBS, fixed with 4% (w/v) paraformaldehyde, and permeabilized with 0.5% Triton X-100. Then cells were incubated with 0.1 μM TRITC-phalloidin to visualize F-actin. Fluorescence imaging was performed in PBS buffer with a confocal microscope (C2, Nikon, Tokio, Japan).

3. Results and discussion

3.1. Effects of TGF-β1 on morphology and mechanical properties of T24 living cell during EMT investigated by AFM

Peakforce tapping operating mode was applied to investigate effects of TGF-β1 on morphology and mechanical properties of T24 living cell during EMT. The high-resolution topography of T24 living cells without TGF-β1 treatment exhibited a cobblestone-like phenotype with a width of 20.5 ± 1.5 μm and a length of 42.2 ± 2.3 μm (Fig. 3A_1, Fig. S1).

Download English Version:

https://daneshyari.com/en/article/10128036

Download Persian Version:

https://daneshyari.com/article/10128036

Daneshyari.com