A NEW DISCRIMINATION METHOD BASED ON BULGE GENERATION BETWEEN CANCEROUS AND NORMAL CELLS

Yu Chang Kim¹², Sang-Jin Park² and Je-Kyun Park¹
¹Department of Bio and Brain Engineering, KAIST, KOREA and ²Energy System Research Division, KIMM, KOREA

ABSTRACT

This paper presents a new and convenient analysis method for discrimination between cancerous and normal cells based on bulge generation upon compression by poly(dimethylsiloxane) (PDMS) membrane deflection in a microfluidic device. The cancerous and normal cells having different contents of cytoskeleton are supposed to be capable of showing something unusual in the bulge generation under excessive deformation. In this study, we have demonstrated the difference of the bulge generation of cancerous and normal cell.

KEYWORDS: Cancerous cell, F-actin, Bulge generation, Peripheral strain

INTRODUCTION

Recently, several techniques such as atomic force microscopy [1] and optical deformability-based microfluidic optical stretcher [2] have been used for cancer cell analysis. However, these sorts of techniques are limited to probing cells under small deformation. In case of small deformation, because cells are neither homogeneous nor isotropic, it is not easy to express the difference between cancerous and normal cells. It has been reported that the amount of F-actin in cancer cells is fewer than that of normal cells [3] and bulges occur at the sites where cytoskeleton becomes detached from the membrane bilayer. In this study, we propose a new discrimination method based on bulge generation between cancerous and normal cells. Here, compressive force is applied to cells through the deflection of the PDMS membrane between control and flow channels. When a cell is excessively compressed, cellular membrane will expand and then small bulges will be appeared on the peripheral cell membrane beyond the allowable strain.

EXPERIMENTAL

As shown in Figure 1a, the microfluidic device is composed of two channels (one for MCF7 and the other for MCF10A cells). For compressing the cells, the PDMS membrane is used as a pressure loading component.

To examine the relationship between the bulge generation and peripheral strain (εₚ), we measured the initial and enlarged diameter of these two types of cells. The strain in the cell peripheral (circumferential) length was calculated by the measurement of the cell diameter as follows:

\[ \varepsilon_p = \frac{l_c - l_0}{l_0} = \frac{d_c - d_0}{d_0} \] (1)
where $l_0$ and $l_e$ is an initial and enlarged peripheral length, respectively, and $d_0$ and $d_e$ is an initial and enlarged diameter, respectively. Here, the enlarged diameter means the central lateral diameter just at the moment of the bulge generation.

RESULTS AND DISCUSSION

After excessive deformation, the bulges generated in MCF7 cells were not evenly distributed on the cell periphery. Contrary to this, the bulges of MCF10A cells showed an even distribution. In addition, the morphologies of bulges of MCF7 and MCF10A cell looked swollen protrusion and tubular protrusion, respectively (Figure 2).

Peripheral strains at the moment of the bulge generation were also 72% in MCF7 and 46% in MCF10A (Figure 3). Since the difference of the bulge generation was explainable through the cytoskeleton transformation, the difference of the amount of cytoskeleton was confirmed through the immunostaining. As shown in Figure 4, the MCF7 having the amount of F-actin lower than MCF10A showed the smaller area.

Figure 2. Comparison of bulge generation between MCF7 and MCF10A cells deformed under compression. Two types of cells showed the tendency to generate different shape of bulges. The generated bulges were also different in distribution. The last column of each cell is the processed images with the sharp edge using ImageJ program.
Figure 3. Comparison between two types of cells, MCF7 (a~d) and MCF10A (e~h), based on the peripheral strains at the moment of bulge generation. (i) The strain of MCF7 is 71.9±15.7% (n=47) and the strain of MCF10A is 46.3±12.1% (n=60).

Figure 4. Fluorescent confocal images of F-actin in two types of cells; (a) MCF7 and (b) MCF10A cells. Scale bars are 20 μm. (c) Comparison of the pixel areas in the stained cytoskeleton. The images of spread and round shape were separately compared for the analysis of the amount of F-actin and tubulin.

CONCLUSIONS

As a result, this study was the first demonstration correlating the bulge generation with the cytoskeleton quantity inside a cell in that a visible change of the external feature reflects an internal alteration such as cytoskeleton transformation. The generality of these findings is yet to be determined. However, the results can be extended to cell markers for making a distinction between cancerous and normal cells through further experimentation with the methods outlined.

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REFERENCES