Single-Molecule Force Spectroscopy study of the interactions between lectins and carbohydrates on cancer and normal cells

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Experimental section

Cell culture

Hela and MDCK cells were bought from Shanghai Institute of Biological Sciences. Hela and MDCK Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) or RPMI 1640 medium on cover slips, respectively. All these mediums were supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were grown in a humidified atmosphere with 5% CO_2 at 37 ^{o}C. Before performing experiments, the cells were rinsed with fresh DMEM for three times to remove the metabolite of cells.

Modification of the AFM tips with lectins

Modification of the AFM tips with lectins was carried out as reported previously. [1] Briefly, the tips were cleaned at O_3 atmosphere in UV-cleaner for 20 min to get rid of the organic contamination. Then the tips were vapor treated with APTES, and reacted with polyethylene glycol (PEG)-crosslinkers in triethylamine (Sigma) and $CHCl_3$. Then

the cantilevers were immersed in 100 μg/mL WGA (Wheat germ agglutinin, from *Triticum vulgaris* (wheat), Sigma) or PMA (pure *Polygonatum mulitiflorum* lectin, from common Solomon's seal, EY Laboratories, Inc.) solutions with $NaCNBH_3$ as catalyst. 1 M ethanolamine was added to passivate the unreacted aldehyde groups. Then the modified tips were rinsed with PBS for two times and stored in PBS at 4 °C until use.

Atomic force microscopy

All the experiments were performed with the Agilent AFM 5500 (Agilent Technologies, Chandler, AZ). AFM imaging was carried out in Acoustic AC (AAC) mode in DMEM at room temperature. The constants of the cantilevers of tips were 0.01 N/m (nominal). The scan rate is 1.5 Hz. The images were recorded as 512 × 512 pixels.

Force spectroscopy was obtained in force-distance mode in DMEM at 37 °C. The spring constants of the Si_3N_4 tips is 0.03 N/m (nominal). The deflection sensitivity of the photo-detector was determined by the slope of the force curves taken on the surface of newly cleaved bare mica. The spring constants of the cantilevers were measured with the thermal noise method in air as described. [2] Thousands of force curves were obtained on various positions on different cells. Blocking experiments were carried out by the addition of 100 μg/mL cognate lectin into the sample cell. The data were processed with MatLab 7.9 (Math Works Inc.).

Fluorescence labeling and imaging

The glucose and mannose in the cell membranes were labeled with WGA and PMA, respectively. 100 μL 100 μg/mL WGA or PMA reacted with 0.1 μL 10 mg/mL cy5-NHS, then the solution was oscillated for 1.5 h in darkness. Unreacted cy5 was filtered with the G-25 SpinTrap (GE healthcare), and then cells were labeled for 1 h. Before imaging, the cells were washed with PBS for three times to remove the redundant dye. The fluorescence images were obtained with the Leica SP2 laser scanning confocal microscopy. Cy5 was excited with the 633 nm laser line on the 100×/1.4 oil immersed objective.

Reference:

1. J. G. Jiang, X. Hao, M. J. Cai, Y. P. Shan, X. Shang, Z. Y. Tang and H. D. Wang, *Nano Lett.*, 2009, **9**, 4489.
2. H. J. Butt and M. Jaschke, *Nanotechnology*, 1995, **6**, 1.