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Detection of carbohydrates on the surface of cancer and normal cells with topography and recognition imaging

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1. The surface morphology of Hela and MDCK cells

In order to confirm that there are galactoses on the surface of cells, the cell membranes were labeled with cy5 conjugated PHA-L, and the fluorescence images captured on Hela and MDCK cells are shown in Fig. S1a and S1b, respectively, which definitely indicates that there are galactoses in both types of cells.

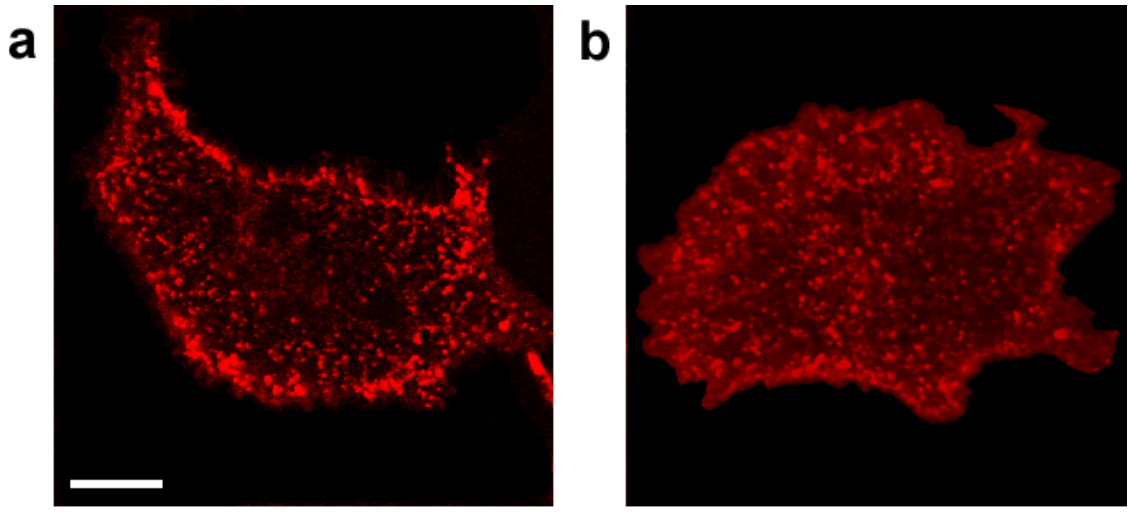

Fig. S1. Confocal fluorescence images. (a) and (b) the fluorescence images of galactose on the surface of Hela and MDCK cells, respectively. The galactose was labeled with cy5 conjugated PHA-L. Scale bar: 10 μm.

2. The scheme of tip modification and the principle of TREC

As the resolution of fluorescence microscopy is limited, we prefer molecular recognition imaging (TREC) to investigate the distribution of carbohydrates at single molecular resolution. The tips were functionalized with PHA-L via a flexible heterobifunctional PEG crosslinker as depicted in Fig. 1a. One PHA-L connects only one PEG crosslinker. The nonlinear stretching characteristic of PEG can make it to distinguish the specific events from the nonspecific. As the PEG is inert in chemical and physical, it makes the PHA-L modified on the tips to reorientate rapidly and freely when the tip is approaching the surface. And the PEG tethered PHA-L modified on the tip can avoid to be crashed. [1] The nonlinear stretching characteristic of PEG can make it easily to distinguish the specific events from the nonspecific. Cells (in which galactoses were shown as yellow spots) were scanned with PHA-L modified tips. The principle of TREC is shown in Fig. 1b. The amplitude of the cantilever is set to be less than the stretched length of the crosslinker. [2] When the galactose sites in cell membranes were scanned, the crosslinker will be stretched in the retraction process of the cantilever. The resulting energy loss will reduce the top peak of the oscillations; thus the recognition signal can be achieved and detected. The raw deflection signal of the cantilever was split by the PicoTREC, the upper and lower parts of each circle were recorded as the recognition and topography, respectively. [3] The recognition process has been confirmed to be highly efficient, specific and reproducible. [2]

3. Experimental section

Cell culture

Hela and MDCK cells were bought from Shanghai Institute of Biological Sciences. Hela was cultured on the cover slides in the Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivate fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. MDCK was cultured in the RPMI 1640 medium supplemented with 10% heat inactivate fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Two types of cells were cultured in a

humidified atmosphere with 5% CO_2 at 37 °C. The cells grew to a confluent monolayer for one or two days.

Functionalization of the AFM tips with lectins

The functionalization procedures were similar as described previously. [4] Briefly, the tips were cleaned in the 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 (9.8 nm in length) in triethylamine (Sigma) and $CHCl_3$. Then the cantilevers were immersed in 100 μg/mL PHA-L (pure Phaseolus valgaris lectin, from red kidney bean, EY Laboratories, Inc.) with $NaCNBH_3$ as catalyst. In the last, 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 and TREC

All the experiments were performed with the Agilent AFM 5500 (Agilent Technologies, Chandler, AZ). The molecular recognition imaging was performed by the Magnetic AC (MAC) mode with the PicoTREC imaging attachment. TREC was carried out with PHA-L modified tips on gently fixed cells in buffer solutions at room temperature. The tips were conjugated on silicon nitride cantilevers (nominal spring constants 0.05 N/m). The scanning rate is 1 Hz. All the images were captured as 512 × 512 pixels. Blocking experiments were performed by the addition of the 100 μg/mL PHA-L into the AFM sample cell. The recognition images were analyzed by the software Recognition Imaging Analysis. The recognition signals were discriminated from the background at the 75% cut-off of the background intensity. [4] The coverage of carbohydrate molecules was calculated by Photoshop software 7.0.1, in which the areas of these spots can be obtained in “image”-“histogram”-“pixel”.

Force spectroscopy was obtained in the force-distance mode in DMEM with modified silicon nitride tips of 0.03 N/m spring constant (nominal) at 37 °C. 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 previously. [5] Thousands of force curves were obtained on various positions on different cells. Blocking experiments were carried out by the addition of 100 μg/mL PHA-L into the sample cell. The data were processed with MatLab 7.9 (Math Works Inc.).

Fluorescence microscopy imaging

The galactoses in the cell membranes were labeled with PHA-L. 100 μL 100 μg/mL PHA-L reacted with 0.1 μL 10 mg/mL cy5-NHS, the solution was oscillated for 1.5 h in darkness at room temperature. 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 unreacted dye. The fluorescent 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-immersion objective.

References:

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