Supplementary Information

Interrogation of drug effects on HeLa cells by exploiting new AFM mechanical biomarkers †

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CCK-8 Assay

HeLa cells prepared as described in "Cell culture" in the "Methods" of the main article were diluted 20-fold in the cell culture media and grown in 96-well (100 μL/well, 1×10^5 cells/mL) plates for 12 h, followed by removing the media and adding 100 μL/well cell culture media containing 0.5% DMSO (control group) or docetaxel solution (experimental group). The cells were then grown for a further 12 h. Afterwards, 10 μL of the CCK-8 reagents were added to each well and let the cells growing for another 2 h. The plates were then transferred to a microplate reader (Epoch, BioTek Instruments Inc., Shoreline, USA) and the OD_{450} value was measured. The data for each condition represents average values taken from three replicate wells performed in three independent experiments.

Western blot assay

The cells were lysed by incubating them in a 6-well plate with lysis buffer (Liankebio, Hangzhou, China) for 15 min in an ice bath and the solution was then collected and centrifuged at 14, 000 × g for 3 min. The supernatant containing proteins was applied onto a 10% SDS-polyacrylamide gel for electrophoresis (SDS-PAGE), in which one lane contained a pre-stained protein ladder (10 kDa -170 kDa, Willget Biotech Co., Ltd., Shanghai, China), and three lanes contained the untreated (control) samples and three lanes contained the docetaxel-treated samples. Following electrophoresis, the proteins were transferred to a poly(vinylidene fluoride) filter membrane (Bio-Rad, Liankebio, Hangzhou, China) and blocked with 5 mL low background blocking solution (5% BSA) for 40 min at room temperature. The primary mouse monoclonal anti-*β*-tubulin antibody (1:5, 000 dilution, Mab1445, Liankebio, Hangzhou, China) was added to the membrane and incubated for overnight at room temperature. After washing with Tris-Buffered Saline Tween 20 (TBST), the membrane was incubated in a secondary horseradish peroxide (HRP)-conjugated Goat anti-mouse immunoglobulin antibody (1:5, 000 dilution, Liankebio, GAM007, Hangzhou, China) for 2 h at room temperature and washed with TBST three times for 10 min each. The secondary antibody was then stained using an

electro-chemiluminescence kit (Ultra ECL Kit, U1421, Liankebio, Hangzhou, China) for 5 min at room temperature, and the bands on the membrane were then photographed by an ECL western blotting analysis system. In this assay, *β*-actin from the cell samples was used as a loading control and the detection procedures are the same as those for detecting the *β*-subunit of tubulin, except for that the primary mouse monoclonal anti-*β*-actin antibody (1:5, 000 dilution, Mab1445, Liankebio, Hangzhou, China) was used, instead of the anti-*β*-tubulin antibody.

The Pincus theory and data fitting

The Pincus theory was originally developed to describe the compression behaviour of a polymer brush grafted on a rigid surface, whereby the length of polymers that behave like a brush can be obtained by fitting the force-distance curve with the Pincus theory.[1] Recently, we extended the Pincus theory and successfully applied it to investigate the brush layer on prokaryotic bacterial cells.[2-5] In the present study, we used the modified Pincus theory to interrogate the brush layer on eukaryotic cell (HeLa cell) surface. The Pincus theory can be written as:

$$F_{loading} = A_p \ln\left(\frac{\delta_L^0}{\delta_L^0 - \delta}\right) \qquad \text{(S1)}$$

where the applied loading force, $F_{loading}$ is a function of the distance, δ. ${\delta_L}^0$ is the onset of linear compliance on the approach curve, approximated as the origin of the apparent nondeformable surface covered by biological polymers.[2] A_p is a numerical pre-factor:

$$A_{\mathrm{p}} \propto \frac{4\pi k_{\mathrm{B}} T N_{\mathrm{B}}}{d^2} \qquad \text{(S2)}$$

where d is the grafted interchain distance of the polymer brush, N_{B} is the number of monomers carrying ionic charge, k_{B} is Boltzmann's constant and T is temperature. A detailed description and derivation of this equation can be found in the Supporting Information of the reference 2.

As shown in Fig. S1, the force profile was plotted on the semi-log scale in order to see the data points more clearly at low load, as is normally done in such data fitting. The onset of the linear compliance region (δ_L^0) was first determined by using a linear fit (black line) to the data on the left side of the force profile. Detailed information can be found in the Supporting Information of the reference 2. The goodness of fit was evaluated by using a prediction band with 95% confidence intervals and more than 95% of the data points fall within the prediction bands (please see below for the prediction bands). Detailed information can be found in the Supplementary Information of reference 3. The point where the force profile deviates from the linear fit was δ_L^0, calculated to be -3.03 μm. This linear fit can be clearly observed from the inset of the figure, which is plotted under the Cartesian coordinate system.

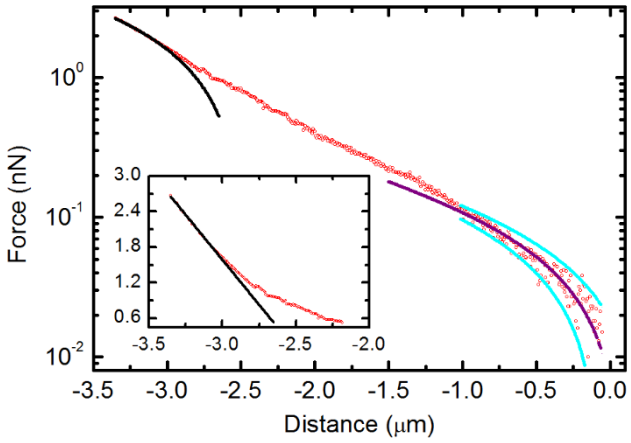

Fig. S1 A typical force profile, plotted on a semi-log scale, for the interaction between an AFM tip with a Hela cell in PBS buffer. The black line shows a linear fit to the force profile to determine the onset of the linear compliance region, δ_L^0, calculated to be -3.03 μm. Afterwards, compression of the cell surface brush was well fitted by the modified Pincus theory (purple line) for indentations up to 1.01 μm (0 to -1.01 μm tip-sample distance). The goodness of Pincus fit was evaluated by using a prediction band with 95% confidence intervals (cyan lines). The inset shows the enlarged linear fit under the Cartesian coordinate system.

Once $\delta_L{}^0$ has been decided, equation (1) can be used to fit the force profile from the right side of the force profile, using the iterative procedure of Levenberg-Marquardt algorithm, coded in-house in Igor Pro (Version 6.04, Wavemetrics Inc., Lake Oswego, USA). The estimates of initial values for unknown variables were restricted in the first instance to physically meaningful values. The parameters that yielded the lines of best fit to the data were selected as those for which chi-squared was minimised. In this example, the force profile over the region (-1.01 μm to 0) has a form typical of polymer brush compression and was well-fitted by the modified Pincus theory, as indicated by the purple line superimposed over this region. The fitted length of the polymer brush is 1.01 μm.

The goodness of fitting was evaluated by using prediction bands (e.g cyan lines in Fig. S1) at 95% confidence interval.[3] The prediction bands were calculated via:

$$\hat{Y} \pm t_{(v,1-\alpha/2)}\sqrt{\sigma^2 + V(\hat{Y})} \qquad \text{(S3)}$$

where $\hat{Y}$ is the predicted value of the model at a given value of the independent variable X, $V(\hat{Y})$ is the variance of a predicted model value, and σ^2 is the sample variance. The function $t_{(v,\ 1-\alpha/2)}$ is the value on a Student's t-distribution with v degrees of freedom. At the 95% confidence level, 95% of the experimentally measured data points should fall within the prediction bands. In each of our fittings, more than 95% of the data points fall within the prediction bands and are reasonably well distributed within the prediction bands.

Notes and references

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