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Nanomechanically Visualizing Drug-Cell Interaction at the Early Stage of Chemotherapy

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ABSTRACT:

A detailed understanding of chemotherapy is determined by the response of cell to the formation of the drug-target complex and its corresponding sudden or eventual cell death. However, visualization of this early but important process, encompassing the fast dynamics as well as complex network of molecular pathways, remains challenging. Herein, we report that the nanomechanical traction force is sensitive enough to reflect the early cellular response upon the addition of chemotherapeutic molecules in a real-time and non-invasive manner, due to interactions between chemotherapeutic drug and its cytoskeleton targets. This strategy has outperformed the traditional cell viability, cell cycle, cell impedance as well as intracellular protein analyses, in terms of fast response. Furthermore, by using the nanomechanical traction force as a nanoscale biophysical marker, we discover a cellular nanomechanical changes upon drug treatment in a fast and sensitive manner. Overall, this approach could help to reveal the hidden mechanistic steps in chemotherapy and provide useful insights in drug screening.

Chemotherapy, involving the use of cytotoxic antineoplastic agents to eradicate tumor cells, remains an important strategy in the overall management of patients with malignant tumors.¹⁻¹⁵ Current chemotherapy is characterized by uptake of the drug into cancer cells, activation of several consequential cellular processes including the signalling of DNA damage, cell-cycle checkpoints and arrest, DNA repair and altered expression of oncogenes or regulatory proteins. A better knowledge of the cellular response to chemotherapy is very important as it provides valuable clues for the rational design of more efficient antineoplastic drugs as well as the development of therapeutic strategies.¹⁶⁻¹⁸ However, the elucidation of the molecular, cellular and physiological processes of chemodrugs involves high costs and long development cycles. Therapeutic effect of most clinically available drugs occurs at the early stages of chemotherapy, and it involves the direct binding of the drug to target proteins and modulating the protein activity, which leads to corresponding cellular or physiological responses. Thus it is of utmost importance to understand drug-cell interactions at the initial stages of chemotherapy.^{11,19} Currently, numerous approaches including cell viability assay, cell cycle analysis, oncogene or regulatory protein expression, or drug-target protein binding detection, have been devoted to identify the molecular mechanism and efficiency of chemotherapeutics. However, these methods encounter a number of limitations including long processing time, expensive equipment requirement, high reagent consumption and difficulty to observe the cellular response in a label-free and non-invasive manner. For example, the colorimetric MTT assay, based on the viable cells' ability to reduce yellowish 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide into purple formazan, was utilized for screening the cancer cell viability upon

chemotherapeutic treatment by detecting the cellular intact mitochondria/metabolic activity, whose activation by chemotherapeutics usually takes more than 16 h, and was not able to reflect the early stages of chemotherapy.

Recent developments in mechanobiology, which focuses on studying the mechanotransduction signalling in mechanical force or geometry dependent cell growth or motility process, have attracted increasing interests.²⁰⁻²⁴ In details, living cells can rapidly sense mechanical forces and convert them into biological responses. Similarly, biological and biochemical signals are known to influence the abilities of cells to sense, generate and bear mechanical forces. Studies on the mechanics of single cells, subcellular components and biological molecules have rapidly evolved during the past decade with significant implications for bionanotechnology and human health.²⁵⁻⁴⁰ This progress has been facilitated by capabilities for measuring forces and displacements with piconewton ($1 \text{ pN} = 10^{-12} \text{ N}$) and nanometre ($1 \text{ nm} = 10^{-9} \text{ m}$) resolutions, respectively, and by improvements in bio-imaging. These advances have provided opportunities for examining the processes responsible for operation of cellular machinery, the forces arising from molecular motors and the interactions between cell and extracellular stimulus (*i.e.* chemotherapeutics). Furthermore, in pharmaceutical research, cell culture based assays are increasingly exploited to bridge the gap between molecular level assays and animal tests, mainly because they can evaluate drug effects on cell proliferation, apoptosis and migration, and provide higher throughput than time-consuming animal experiments. Mechanobiology focuses on the way that physical forces and changes in cell mechanics

contribute to development, physiology, and disease. It is a very promising field to overcome these limitations, because mechanotransduction, the mechanical molecular mechanism by which cells sense and respond to stimulus, is a very powerful tool to visualize the drug-cell interaction. For example, chemotherapeutic emodin,⁴¹ cytochalasin D,⁴² and latrunculin B,⁴² which possess the ability to disrupt cellular mechanotransduction related actin filament and may provide a quantitative reflection of the cellular dynamics during the therapeutic process. Herein, we propose that *in situ* cellular nanomechanics may possibly be a suitable downstream cellular response indicator for non-invasive evaluation of drug efficacy in chemotherapy. This successful trial may shed light on the mechanistic actions of the anti-cancer drug within cancer cells, and assist in the development of assays that can quantitatively assess the efficacy of anti-cancer drugs.

In order to test our hypothesis, nanomechanical traction force (or cell traction force, CTF), generated by cells with the ability to probe, push or pull on the surrounding extracellular matrix, is chosen as a typical indicator for cellular nanomechanics to reflect the drug-cell interaction. This nanomechanical force is chosen because it reflects cell response at the nanoscale (typically in terms of nN) and has great potential in directing numerous cellular processes, including spreading, migration, differentiation, and even apoptosis.^{41,43-46} Furthermore, the non-invasive quantitative characterization of these cellular nanomechanical forces in terms of their direction and magnitude may be helpful towards understanding or even predicting physiological and pathological events in cells or tissues. For example, the mechanical loading of

heart muscle cells can be an indicator of healthy or diseased status, as heart failure is normally due to the contractility loss of heart muscle cells.^{47,48}

Herein, we report that this nanomechanical traction force, albeit small, is sensitive enough to reflect the early cellular response to chemotherapeutical molecules in a real-time and non-invasive manner, which outperforms the traditional cell viability, cell cycle, cell impedance as well as intracellular protein analyses. As a model system, HeLa cells (a human cervical cancer cell line) and a chemotherapeutic drug paclitaxel (PTX) suitable for ovarian carcinoma treatment were chosen. Further investigations through the use of PTX-analogue with reduced microtubule-binding ability as negative control and immuno-staining of cytoskeleton of HeLa cell after PTX treatment, indicate that the change of cellular nanomechanics at the early stage of chemotherapy is caused by PTX with the ability to bind to and re-organize microtubules inside the cancer cell, which has also been confirmed by using molecular simulations. Even more interestingly, we have validated that cellular nanomechanical mapping is sensitive enough to reveal more complex drug-cell interactions, using nocodazole as another chemotherapeutic molecule that depolymerize microtubule, is applied. Overall, these results provide useful insights into chemo-mechanical signal transduction during cancer therapy and can potentially help in research on chemotherapeutic drug screening and development.

Results and Discussion

In a typical example, we proposed a strategy for the quantitative mapping of real-time cell traction stress (cellular nanomechanical force per area) exerted by cancer cells in response to

the uptake of anticancer drugs, which are cultured on ultrasoft polydimethylsiloxane (PDMS) substrate embedded with a single layer of fluorescent beads as position markers (Figure 1). With the elastomer compliance quantified independently using controlled deformation, nanomechanical force was visualized by employing a computation for the relationship between marker displacements. This design provides a powerful combination of capability and efficiency in permitting a quantitative determination of nanomechanical force magnitudes and directions across an entire cell, with essentially local force magnitude and temporal resolution (on the order of nN and minutes, respectively). The nanomechanical forces exerted by the adherent cells would generate small deformations in the soft PDMS substrate and result in slight fluorescent bead displacements, from which the traction forces exerted by the cells could be calculated. This concept was demonstrated in the tracking of real-time cellular nanomechanics changes in HeLa cells during its initial uptake of the chemotherapeutic molecule paclitaxel (PTX) (Figure 1c). PTX was chosen as the prototypical anticancer drug due to two reasons; it is one of the most successful chemotherapeutic drugs for the treatments of ovarian and breast carcinomas. It possesses the ability to inhibit mitosis through the formation of the abnormal microtubule bundles, which are among the most successful targets for anticancer therapy and are key cytoskeleton to regulate cellular nanomechanical force.^{31,49} PTX binds with low affinity to the free β -tubulin molecules, but bind strongly to the β -tubulin along the microtubule to form an abnormal microtubule without de-polymerization ability. However, the detailed process and underlying mechanism for the binding of PTX to tubulin and its subsequent conformational change towards affinity enhancement between neighbouring tubulin molecules remains unclear.⁵⁰

As the microtubule is a key component of the cytoskeleton, which is closely related to cellular mechanical property,⁵¹⁻⁵³ we hypothesized that temporal-spatial interaction between chemotherapeutic drug PTX and microtubule could be quantitatively revealed by using real-time cellular nanomechanical stress mapping of human cancer cell. In addition, we expected that the process could also be an example to demonstrate the successful conversion of intracellular event to detectable biomechanical signal in the form of cell traction kinetics.

Prior to the real-time cell traction stress mapping, the elastic PDMS substrate embedded with fluorescent beads must first be fabricated. In our design, ultrasoft PDMS (1:60 w/w of crosslinker to base ratio) was the material of choice due to its biocompatibility, resistance to swelling, optical transparency down to 260 nm, as well as a Young's modulus of 15 kPa, which is close to the elastic moduli of most tissues in the order of 1-50 kPa.⁵⁴⁻⁵⁶ Our PDMS substrate was embedded with only a single layer of fluorescent beads (0.1 μm FluoSpheres[®] microspheres) at the interface, instead the traditional method of randomly mixing fluorescent beads within the PDMS,^{57,58} as such a design would render the fluorescent beads on the same focal plane (Figure 1b). This would greatly simplify the beads tracking process and reduce the error arising from unfocused fluorescent beads. Not only that, as nanomechanical traction force decays steeply in the z direction, our design could possibly minimize errors in the calculation of cell traction force by negating the need to consider the z-position of embedded fluorescent beads positioned out of the focusing plane.⁵⁹

Furthermore, the ability to visualize both the substratum displacements and molecular components within the cells, through real-time microscopy, provides approaches for investigating potential relationships between force transmission and the dynamics of components involved in cell–substratum adhesion. To clarify the chemo-nanomechanical effect of PTX on HeLa cells, cell culture medium without any drug and with PTX-analogue without microtubule binding ability (the chemical structure and characterization by ^1H -NMR and mass spectrometry are shown in Figures S1-S4) were first used as negative controls. The control experiment was conducted by using single HeLa cell without contacting adjacent cells to ensure no effect from cell-cell interaction. The cell with continuous treatment of culture medium without any drug or with PTX-analogue ($1\ \mu\text{M}$) for a short incubation period (1 h each) showed strong cell traction stress at the two ends of HeLa cells (Figure 1c, which showed the DIC image of HeLa cell as well as the corresponding underneath traction stress mapping in pseudocolor indicating regions of traction stresses with different magnitude). The cellular nanomechanical traction stress mapping showed that traction stresses were larger at the edge of HeLa cell, indicating larger traction stresses at the actin-rich pseudopodia than the areas surrounding the nucleus.^{57,60} Interestingly, the HeLa cell exhibited stable net nanomechanical force upon incubation with culture medium containing either no drug or PTX-analogue ($1\ \mu\text{M}$) (Figure 1d), indicating that normal cell behaviour was not disturbed in control experiments.

After confirming the stability of net CTF in the control experiment, the tempo-spatial effect of chemotherapeutic PTX on HeLa cell was then explored through the introduction of

medium containing 1 μ M of PTX. Figure 1c and Figure 1d showed that during the first 15 min of drug treatment, the HeLa cells exhibited similar cellular behavior when compared with control experiment and PTX-analogue. Beyond 15 min, the HeLa cell exhibited decreasing cellular nanomechanical force (~ 200 nN), attributable to the chemical stimulus of PTX. Prior to treatment with PTX, the HeLa cell tended to increase the traction stress beneath its right pseudopodia and decrease the traction stress beneath its left pseudopodia, which suggested that the cell was trying to migrate to its right (the first 2 h in Figure 1c). However, upon the addition of PTX, the behaviour of the HeLa cell was changed, as shown by the decrease in detectable traction stress at its right pseudopodia (Figure 1c). Such a phenomenon represented a fast cellular response, in term of nanomechanical force, to chemotherapy and is indicative of the ability of PTX to inhibit microtubule dynamics and cell migration. Our result also indicated that PTX could limit the number of microtubule reaching cellular lamellipodium, which in turn inhibited cell migration.⁶¹⁻⁶³ This abnormal cellular behaviour observed through real-time cellular nanomechanical force measurement could also be explained by drug-cell interaction, leading to the inhibition of microtubule depolymerization and subsequently impaired cell migration.⁶⁴ To ensure that the observed cellular nanomechanical force change was applicable for all HeLa cells exposed to PTX, the average of net CTFs collected from six cells of similar shape was obtained. As shown in Figure 1d, the HeLa cells exhibited a significant decrease in cellular nanomechanical force when the PTX treatment time was increased beyond 20 min, which was not observed in the control experiments. HeLa cells exhibited detectable cellular nanomechanical force changes within the first hour of chemotherapy, which suggests that cellular nanomechanics

could possibly be a fast and sensitive indicator for cellular physiological status. Moreover, the nanomechanical force of HeLa cell also displayed dose-dependency on PTX (Figure 1e). The results also showed that HeLa cells exhibited greater nanomechanical force reduction within same treatment time when the concentration of PTX was increased, which can be attributed to the stronger interaction between the HeLa cells and PTX at higher PTX concentrations.

To illustrate the advantage of using cellular nanomechanics to quantify early drug-cell interaction, we compared its effect with other traditional approaches such as MTT assay, western blot and flow cytometry (FACS) analysis. First, using colorimetric MTT assay, HeLa cells showed PTX dose-dependent viability ($IC_{50} \cong 1.2 \mu M$) when cells were treated with PTX for 24 h, which indicated that PTX is an effective anti-tumor agent. In contrast, the PTX-analogue exhibited low therapeutic effect as the viability of the HeLa cells remained high (89%) even at high PTX-analogue concentration (5 μM). However, the early stage (1 h) of drug-cell interaction did not induce significant reduction to the viability of HeLa cells (Figure 2a). This result is understandable as MTT relies on the intact mitochondria/metabolic activity of cell, and the activation of the mitochondrial apoptotic pathway by PTX usually takes more than 16 h.⁶⁵ Immunoblotting assays showed cell growth inhibition by PTX as evidenced by PARP cleavage only after 24 h. PARP cleavage was not detected at the early stage of PTX treatment in HeLa cells (Figure 2b), indicating the ineffectiveness of western blot to detect early drug-cell interaction. Finally, FACS analysis using bromodeoxyuridine (BrdU) and propidium iodide (PI) showed that PTX arrested cell proliferation (Figure 2c). However, this only occurred at their G2

phase after 24 h, rather than at the early stage of drug-cell interaction. Finally, cell-based screening assay based on real-time impedance electronic sensing was applied. Although the drug-cell interaction could be easily quantified by this cell-based assay when PTX concentration was above 1 μM , our results showed that the detected cell index values were similar to that of control when PTX concentration was 0.2 μM . This strongly suggests that our cellular nanomechanics based approach could serve as a more sensitive tool for the detection of drug-cell interaction (Figure 2d). This may be due to the fact that cell impedance changes detected by the cell electronic sensing system relied on the cell contact area on the substrate as well as cell-cell interactions. However, such changes in contact area were insignificant compared to the reconstruction of cytoskeleton at low PTX concentration, as elucidated by cellular structure analysis in the later part of this manuscript (Figure 3). Lastly, the western blotting of β -tubulin and β -actin within the HeLa cells with PTX treatment showed that the key cytoskeleton components remained unchanged (Figure 2e). In summary, comparison of the various approaches to quantify the early drug-cell interaction (1 h), such as MTT cell viability, western blot, cell cycle analysis and impedance-based cell electronic analysis, the nanomechanical traction force changes of HeLa cells became detectable after 5 min and was obvious after 20 min (Figure 1e), which indicated that cellular nanomechanics might serve as a sensitive indicator to reflect PTX-HeLa cell interaction.

Intracellularly, actomyosin-microtubules are in a tightly regulated dynamic balance. Contractility is generated as the actomyosin motor proteins slide along the actin filaments that

are transmitted to the ECM *via* the focal adhesion proteins.⁶⁶ Conversely, microtubules serve as compressional elements to counteract the contractility and this dynamic balance thus prevent catastrophic collapse of the cell structure. It is *via* these antagonistic roles between filamentous actin and microtubules that intracellular tensile homeostasis is achieved. Therefore, any perturbation to actin or microtubules stability will increase the overall contractility or nanomechanical traction force of the cells. Hence, to understand the mechanism of PTX-induced CTF dynamics, cytoskeleton immuno-staining was applied to visualize the chemo-mechanical effect of PTX on HeLa cells during the early stage of chemotherapy. As shown in Figure 3a, HeLa cells without any drug treatment comprised of microtubules and actin filaments uniformly aligned across the cell. PTX-treated HeLa cells exhibited no obvious change in actin structure but dense bundles of microtubules coursing around the nucleus and out into the cytoplasm was observed even after 1 h PTX treatment. Microtubules assembly was less obvious in HeLa cells treated with same concentration of PTX-analogue (Figure 3a). These results were consistent with previous reports that the treatment with PTX could induce cytoskeleton reorganization and collapse of the intermediate filament network, culminating in cell morphology change.^{51,67} The accumulation of microtubules distributed in extranucleus region with the area of half nucleus was quantitatively measured, and dramatically enhanced dense microtubules relocation with a value around 78% was observed in PTX-treated HeLa cells, contrasting sharply with the 35% calculated for control sample and 43% for PTX-analogue treatment (Figure 3b). The PTX-treated cells also showed reduced spreading, as reflected by their smaller average surface areas ($583 \pm$

74 μm^2) relative to that of untreated cells ($811 \pm 91 \mu\text{m}^2$), which indicated a reduced ability to maintain cell shape. Taken together, these data suggest that CTF changes are due to the chemotherapeutic (PTX) modulation of structure-function dynamics of the microtubules.

To support our data that CTF showed significant change once the microtubules were targeted by anti-cancer drug, molecular dynamics simulations were conducted using PTX as a model system. The obtained results indicated that PTX gave rise to long-range allosteric changes in the β -monomer of microtubule (β -tubulin) to render a marked increase in (β -tubulin dynamics (Figure 3c-d). Figure 3d showed the root-mean-square fluctuation (RMSF) for both apo- (red) and PTX-bound (green) β -tubulin, which were similar to previous reports.⁶⁸ A 10 ns simulation was then conducted for the bound and unbound β -tubulin. However, only 5 ns of the trajectory was used for bootstrap RMSF analyses as the potential energy of the system stabilizes after 5 ns. Interestingly, the RMSF plots for T1 to T5, H1-S2, H6-H7, M and H11 regions showed the most significant difference between apo- and PTX-bound β -tubulin⁶⁸. Other than the M-loops, all the other regions identified (*vide supra*) showed greater fluctuations in the PTX bound β -tubulin than in the apo-bound β -tubulin, indicating the increase in tubulin flexibility caused by the binding of PTX to β -tubulin, which have significant implications for the overall conformation and increased strength of the microtubule. Taken together, the immunostaining and molecular simulation reflect that intact actin behaviour as well as increased flexibility and strength of microtubule induced significant change in cellular nanomechanical traction force, which visualized the therapeutic effect of PTX in a non-invasive way.

The above experiments provided strong evidence that nanomechanical force played importance role in revealing the specific microtubule-targeting chemotherapeutics' molecular mechanism, in a fast, sensitive and non-invasive manner. Different molecular mechanisms have been described for chemotherapeutics with similar microtubule targeting ability. Thus, we examine if our nanomechanical approach was sensitive enough to distinguish this important difference. To this end, we investigate the effect of another anti-cancer drug nocodazole using cellular nanomechanical mapping. Nocodazole disrupts the intracellular microtubules *via* a different mode of action from microtubule-stabilizing PTX. Although, bright field image of HeLa cell treated with nocodazole showed the similar cell shrinkage to PTX treatment (Figure S7), quantification of the average cellular nanomechanical force by individual cells confirmed a significant increase upon nocodazole addition (10-50 nN) and a following decrease (20-250 nN) with longer incubation (Figure 4a). The reason of this interesting behaviour might be due to the ability of nocodazole to depolymerize the microtubule which is a rigid structure with the ability to absorb some forces from a contractile actin cytoskeleton at the initial stage.⁶⁹ In addition, the loss of intact microtubule network may also initiate global cytoskeletal remodelling and focal adhesion maturation *via* the Rho GTPase signalling cascade. This would in turn induced the formation of stable adhesion to the underlying substrate by the cells that may resist cell migration. Further incubation of nocodazole might lead to complete loss of intracellular rigid microtubule, cellular shrinkage, and loss of focal adhesion, which finally led to the decrease in CTF (Figure 4b). Furthermore, the transition points of CTF shifted to longer incubation time with increasing nocodazole concentrations, indicating CTF could sensitively reveal drug-cell

interaction. In short, the result supported our hypothesis that cellular nanomechanical mapping is a useful tool to quantitatively characterize early drug-cell interactions, and to distinguish the different mechanistic action of different chemotherapeutic.

Conclusion

In summary, by nanomechanical mapping of HeLa cell *via* cellular nanomechanics quantification, we were able to characterize the tempo-spatial cellular biophysiological event such as intracellular chemotherapeutic drug interaction with cytoskeleton and to convert chemotherapeutic stimulus to biomechanical signal, which was proven to be more sensitive than cell viability assay, cytoskeleton component or cell cycle analysis at the early stage of chemotherapy. The results demonstrated that cellular nanomechanics monitoring could serve as a non-invasive approach to study *in situ* drug-cell interaction without introducing foreign chemical assays. The success in revealing the chemo-mechanical relationship suggests that the cellular nanomechanics mapping could be utilized as a useful tool to study the mechanistic effect due to chemotherapeutic action of anticancer drug and could be helpful to understand the intracellular molecular interaction in cancer therapy.

Experimental Methods

Preparation of elastic PDMS substrate embedded with fluorescent beads. To construct the elastic substrate for cell traction force measurement, glass coverslips were chemically modified before casting the fluorescent beads. Briefly, coverslips were rinsed with

acetone and isopropanol and further cleaned by oxygen plasma before being placed in a vacuum desiccator containing a small droplet of 1H,1H,2H,2H-perfluorooctyltrichlorosilane for 30 min. The coverslips were taken out from the desiccators and 100 μ L of diluted yellow-green FluoSpheres[®] microspheres (0.1 μ m, carboxylate-modified, 0.1 vol %, Molecular Probes) aqueous solution was utilized to cast a single layer of fluorescent microspheres on the glass coverslips. Secondly, Thin film of PDMS were generated by placing 15 μ L of PDMS mixture (before curing, 1:60 w/w of crosslinker to base ratio) on the fluorescent microspheres coated glass coverslips. One layer of Scotch tapes was applied as a spacer before putting the other bare glass coverslip on the top of fluorescent microspheres coated glass coverslips. The PDMS droplet was flatten under the weight of the upper coverslip. The entire assembly was left undisturbed for 5 min and then placed in 37 °C incubator overnight before carefully peeling off the upper bonded PDMS thin film with fluorescent microspheres on its top. Only samples with well-dispersed beads were used in the study (Figure S5). To promote cell attachment to PDMS substrate, PDMS film after 30 min UV sterilization was incubated with collagen solution (50 μ g/mL in 0.02 M acetic acid for thin coating) for overnight before further rinsing by using PBS (pH 7.4) solution, and then left in 4 °C fridge sealed by alumina foil before use.

Cellular nanomechanical force calculation method. Calculation of nanomechanical force, stain energy (forces multiplied by displacements), and polarity (ratio between force along principle axe and force along axe which is vertical to principle one) were based on the properties of PDMS substrate and fluorescent beads displacements, which was similar to the technique

described by Dembo and Wang.⁷⁰ The cell traction mapping was based on the DIC image of HeLa cell on modified PDMS substrate and the corresponding traction stress mapping in pseudocolor, which indicated regions of traction stresses (dark blue to light pink according to stresses from low to high). All computational work, including image processing, was performed with Matlab. Briefly, the traction force at discrete point $\vec{f}_i$, located at the position (x_i, y_i) was calculated based on the following formulation: $\vec{u}_t(x, y) = \sum_{i=1}^n \vec{G}(x - x_i, y - y_i) \vec{f}_i$, where $\vec{G}$ denoted the Greens' tensor and $\vec{u}_t$ denoted the experimental displacements of fluorescent beads at position (x_i, y_i) . The overall force of the cell (F) is an integral of the traction field magnitude over the area, $F = \int \int \sqrt{T_x^2(x, y) + T_y^2(x, y)} dx dy$, where $T(x, y) = [T_x(x, y) + T_y(x, y)]$ is the continuous field of traction vectors defined at any spatial position (x, y) within the cell.

Cell culture. HeLa (human cervical cancer cells) cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mg penicillin, 100 μ g/mL streptomycin at 37 °C and 5% CO₂. DMEM medium was purchased from Gibco BRL (Gaithersburg, MD). For HeLa cell with drug treatment, 2 mM of PTX-analogue or PTX stock solution in dimethyl sulfoxide (DMSO) was prepared. 1 μ M of PTX-analogue or PTX solution was prepared by diluting the stock solution in serum-supplemented culture medium (1:2000 dilution) at the time of usage.

Real-time cellular nanomechanical force measurement. The cellular nanomechanical stress mapping was achieved *via* the following steps: (a) HeLa cells were plated onto the elastic

PDMS substrate embedded with fluorescent beads; (b) fluorescence photographs were taken of the fluorescent beads as “force-loaded” images; (c) the HeLa cells were removed and fluorescence photographs were again taken at the same place as “null-force” images; (d) the areas of fluorescent beads displacement were identified by comparing the images before and after cell removal; and (e) mapping of the cellular nanomechanical traction stress of the cells was conducted through the combination of fluorescent beads displacement mapping, computation software, and elasticity theory.

Confocal microscopy and time-lapse imaging. For live imaging of the cells on PDMS substrate, the samples were viewed on a Leica TCS SP5 laser scanning confocal microscope (Leica Microsystems, Mannheim, Germany) equipped with stage top incubator (Tokai Hit, INUB-WELS-F1 series, 37 °C and continuous pumping in of 5% CO₂). The images were recorded by using a 63×/1.40 NA oil-immersion objective lens with scanning resolution of 2048×2048 pixels. The fluorescent beads were excited by using 488 nm (Argon laser) and their emission was collected in the range of 500 nm to 600 nm. Moreover, the differential interference contrast (DIC) images were also recorded to indicate the location and shape of cells.

Molecular dynamics simulations. The molecular dynamics simulation is similar to reported⁶⁸. For parameterization of PTX, the molecular geometry of the PTX structure from the PDB id: 1JFF was optimized with *ab initio* Hartree Fock calculations at the 6-31G* level using Gaussian 09. For molecular dynamics (MD) simulations, energy minimization was firstly conducted before the system heating from 10 K to 300 K using Langevin thermostat. After that,

the system was equilibrated and another 5 ns of production run was further conducted for structure analysis. Structures were extracted every 5 ps from the 5 ns trajectory to obtain 1000 snapshots for subsequent analysis. For bootstrap root mean-square fluctuations (RMSF) analysis, we applied bootstrap analysis to provide a statistical measure for any differences in the RMSF measurements between the apo- and PTX-bound simulations. We first determined the number of independent points in our MD trajectories by looking at the autocorrelation of root mean-square deviations of the structures. To calculate the RMSF, 10 random points were selected, with replacement, from the 5 ns production run and this process is repeated 200 times. The average RMSF were then calculated for the apo and holo system and the RMSF for the beta-tubulin was plotted. Using these values we applied a student's t-test to the data and identified contiguous regions of amino acids that showed significant differences at a significance level of $p < 0.005$.

ASSOCIATED CONTENT

Supporting Information. Supplementary information includes the synthesis and structure of PTX and PTX-analogue, preparation of elastic PDMS substrate embedded with fluorescent beads, cellular experimental methods, molecular simulation approaches, cell traction force mapping of HeLa cells treated with nocodazole or doxorubicin, and a typical fluorescent bead displacement mapping of HeLa cells. Supplementary information accompanies this paper is available free of charge *via* the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

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Figure 1. Design of using cellular nanomechanics as a biomechanical marker to detect drug-cell interaction at the early stage of chemotherapy. (a) Schematic of observing cellular nanomechanical dynamics induced by anticancer drug stimulus. (b) Schematic of the design of cellular nanomechanics measurement by applying ultrasoft elastic PDMS substrate with Young's modulus of 15 kPa embedded with one layers of fluorescent beads. Elastic substrate was deformed upon cellular nanomechanics was applied, and its deformation could be quantified by its embedded fluorescent tracers for real-time cellular nanomechanical stress mapping. (c) Real-time cellular nanomechanical stress mapping during continuous stimulation of single HeLa cervical cancer cell with culture medium (DMEM, 1 h), PTX-analogue (PTX-analogue, 1 μM, 1 h), and PTX (PTX, 1 μM, 2 h), which indicated that CTF could be utilized to sense drug-cell interaction. (d) Quantification of Time-lapse cellular nanomechanics of HeLa cell subjected to different chemical stimuli as shown in (c). (e) Mean cellular nanomechanical force of HeLa cells

(n = 6) showed significant changes upon treatment with differently concentrated PTX. *, $P < 0.05$.

Figure 2. Cellular nanomechanics measurement outperforms the traditional cellular analysis in detecting early stage of cancer cell behaviour when treated with anti-cancer drug. (a) Cell viability test by using MTT assay when cells were treated with differently concentrated PTX for 1 h and 24 h. The cell viability results showed that drug-cell interaction was not detectable during the first one hour. *, $P < 0.05$. (b) The apoptotic effects of PTX in HeLa cells. Cells treated with PTX (0.2 μ M) for 1 h or 24 h were analyzed by PARP cleavage, indicating that the apoptosis of HeLa cells was visible only with long time incubation. (c) Cell cycle analysis identified that drug-cell interaction was only quantifiable after 24 h, rather than at its early stage. Treated HeLa cells were pulse-labelled with BrdU, fixed and double stained with propidium iodide (PI) and BrdU-FITC. The figures show a bivariate analysis of total DNA content using PI against BrdU incorporation (BrdU-FITC), to calculate the percentage of cells in each phase of the cell cycle. (d) Drug-cell interaction revealed by an impedance-based, real-time cell electronic sensing (RT-CESTM) system (Roche). The cell indexes characterized by this RT-CES system indicate that drug-cell interaction was not detectable during the first one hour, when the PTX concentration was 0.2 μ M. However, it became visible when the PTX concentration increased to above 1 μ M within first one hour or after 24 h incubation. (e) Western blot of β -tubulin and

β -actin shows that the intracellular microtubule and actin cytoskeleton amounts did not change during drug-cell interaction.

Figure 3. Immunostaining of cytoarchitecture of HeLa cells indicates that cellular structure of microtubule changes during PTX-cell interaction, which is corresponding to cellular nanomechanics change. (a) HeLa cells were exposed to cell culture medium as control, 1 μ M PTX-analogue and 1 μ M PTX for 1 h, indicating the microtubule condensation to nucleus during the drug treatment. Microtubules (anti- α -Tubulin-Alexa 488[®]), actin filaments (Alexa Fluor[®] 594 phalloidin) and nuclei (DAPI) were respectively shown in green, red and blue fluorescent color. And differential interference contrast (DIC) images were also shown. Scale bar, 30 μ m. (b) Statistical study of microtubule accumulation around nuclei by fluorescence image processing.

The results show that, for cells with PTX treatment, microtubule accumulated in extranucleus region with are of half nucleus are almost twice compared with cells without any treatment or with treatment of PTX-analogue (*, $P < 0.05$ by student t-test, $n = 20$). (c) Depiction of the regions distal to the PTX binding site that show changes in dynamics when PTX binds. Nine regions in color have the most obvious difference between apo- and PTX-bound β -tubulin. Colored in yellow are the T1–T5 loops as well as a portion of H11 that show a significant increase in dynamics when PTX binds. These loops make up the nucleotide binding site in β -tubulin. Colored in red are the H6–H7 and H1-S2 loops, rather than M loop, also exhibit dynamics increase after binding. (d) Root mean-square fluctuations for apo- and PTX-bound β -tubulin. The RMSF plots for the apo- and PTX-bound structures show very similar behavior over much of the protein; however, nine regions exhibited a statistically significant difference in dynamics when PTX was bound. These areas are highlighted by the gray bars and are shown in detailed plots. The apo simulation is shown in red, the PTX-bound simulation is in green, and the secondary structure of β -tubulin is illustrated at the bottom of the full RMSF plot. (e) A proposed mechanism for cellular nanomechanics change upon PTX addition is shown. PTX binds along the interior surface of the microtubule (a microtubule cut away is shown), increasing its flexibility, leading to microtubule redistribution, as well as its strength and causing cellular nanomechanical property changes.

Figure 4. Cellular nanomechanical force measurement in detecting early stage of cancer cell behaviour when treated with the other model anti-cancer drug nocodazole. (a) Mean nanomechanical force of HeLa cells ($n = 6$) showed an increasing (highlighted by arrows) following decreasing trend upon treatment with differently concentrated nocodazole, which is significantly different from PTX. *, $P < 0.05$. (b) HeLa cells were exposed to cell culture medium as control, 0.2 μ M nocodazole for 1 h and 24 h, indicating the microtubule depolymerization during the drug treatment. Microtubules (anti- α -Tubulin-Alexa 488[®]), actin filaments (Alexa Fluor[®] 594 phalloidin) and nuclei (DAPI) were respectively shown in green, red and blue fluorescent color. And differential interference contrast (DIC) images were also shown. Scale bar, 30 μ m. (c) A proposed mechanism for cellular nanomechanics change upon nocodazole addition is shown. Nocodazole depolymerize the microtubule which is a rigid structure with the ability to absorb forces from a contractile actin cytoskeleton at the initial stage. Further incubation of nocodazole might lead to complete loss of intracellular rigid microtubule, cellular shrinkage, and loss of focal adhesion, which finally lead to the decrease of cellular nanomechanical force.