High-throughput DNA tensioner platform for interrogating mechanical heterogeneity of single living cell

Xinxin Hang, Zaizai Dong, Lingqian Chang
Key Laboratory of Biomechanics and Mechanobiology (Ministry of Education), Beijing Advanced Innovation Center for Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China.
lingqianchang@buaa.edu.cn

Mechanical regulation provides the cells with versatile modes to avoid the environmental stimulations that are detrimental for cell development[1]. The heterogeneities among individual cells lead to different cellular behaviors and responses against the stimulation[2]. In given scenarios, abnormal regulations of mechanical force in specific cell subtypes may introduce disorders to the organism, such as tumorigenesis[3], drug resistance[4], and metastasis[5]. The understanding of the mechanical properties at the single-cell level is an important index to explore the initiation and development of diseases, and further contribute to the advancement of treatment strategies. The shortcomings of conventional methods, including force resolution and cellular throughput, make them less accessible to mechanical-heterogeneity at single-cell level.
In this work, we propose a DNA tensioner platform that achieved both strengths of high-throughput (tens of thousands of cells) and pN-level resolution, and enables to mapping the mechanical force distribution over the single cell (**Figure 1a-1b**). The platform is based on a microfluidic chip patterned with a high-throughput microwell array (> 10,000 microwells/chip) that culture single living cells in each addressable well, allowing for real-time and long-term monitoring (**Figure 1c**). On the bottom of the microwell, we designed a 'hairpin-structured' DNA tensioner that can anchor to the cell membrane. Once the cell mechanical force opens the hairpin, fluorescence signals on the tensioner are recovered, forming an indicator to the cellular force (**Figure 1d**). By using the platform, one can identify enhanced mechanical forces of drug-resistant cells as compared to their drug-sensitive counterpart (**Figure 2a-2e**), and mechanical differences between metastatic tumor cells in pleural effusion and non-metastatic histiocytes. Further genetic analysis traces two genes VEGFA and MINK1 that may play deterministic roles in regulating mechanical heterogeneities (**Figure2f-2h**). In view of the ubiquity of cells mechanical forces in cellular microenvironment (ECM), this platform showing wide potentials to establish links of cellular mechanical heterogeneity to genetic heterogeneity.

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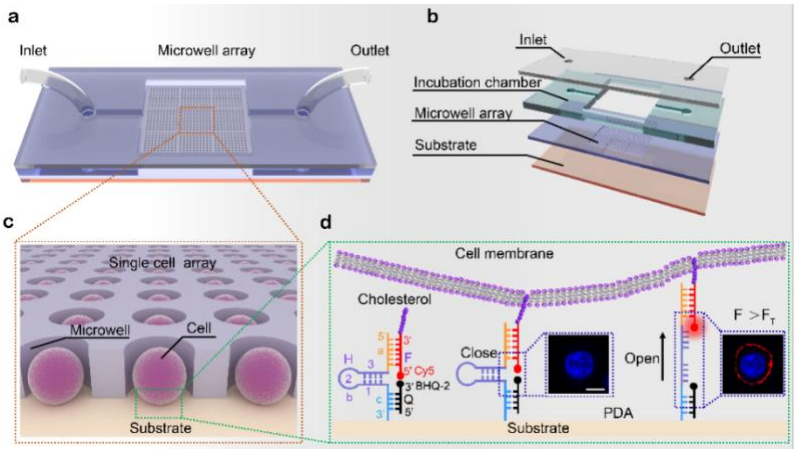

Figure. 1 Principle of the DNA tensioner platform. (a) Schematic illustration of the high-throughput DNA tensioner platform. (b) Layered assembly of the microwell array chip. (c) Schematic illustration of the single cell array. (d) Schematic structure and working principle of the DNA tensioner.

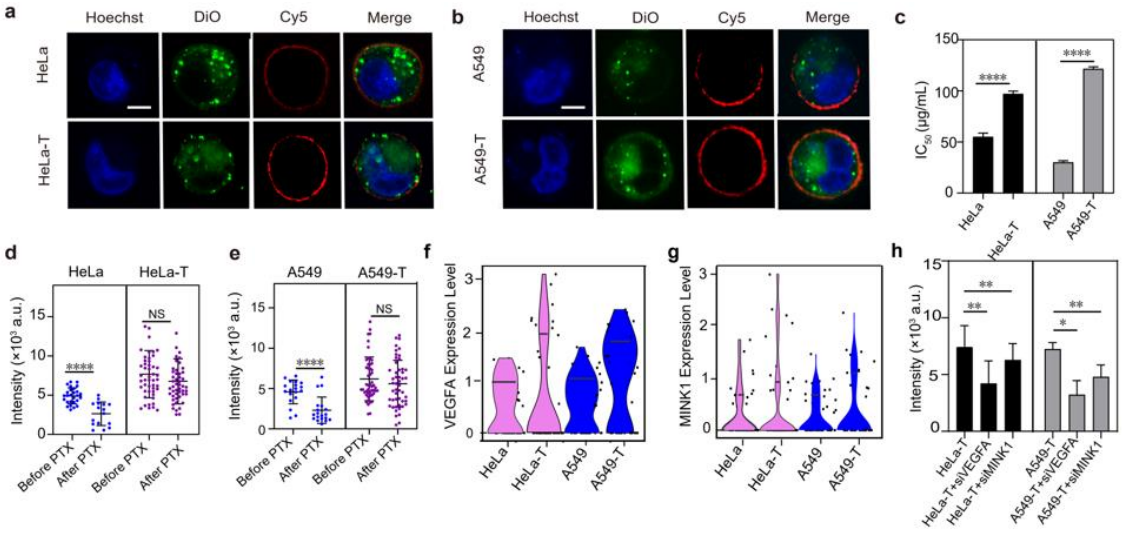

Figure. 2 Single-cell mechanical force analysis for drug-resistant tumor cells. (a) and (b) Representative fluorescence images of PTX-sensitive cells and PTX-resistant cells detected by the DNA tensioner platform. Scale bar: 10 μm. (c) The half-maximal inhibitory concentration (IC_{50}) of PTX drug. (d) and (e) The changes of mechanical force of cells with the incubation of PTX. (f) and (g) The expression levels of VEGFA and MINK1 RNA in cells. (h) The fluorescence intensity of HeLa-T and A549-T before and after treated with siVEGFA and siMINK1. The data indicate mean ± standard deviation (SD) from three experiments. All data are from fifty single cells and are presented as mean ± standard deviation (SD). NS, no significant difference. **, P value < 0.01，****, P < 0.0001.