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Study Piezo1 Localization and Transportation Dynamics by Light-sheet Microscopy

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Abstract: The mechanosensitive ion channel piezo1 plays an important role in mediating varied cellular signaling. It is hypothesized that the diverse functions of piezo1 could be depending on its subcellular localizations. Here we demonstrated that the activation of piezo1 can change its subcellular localization as a feedback by using light-sheet microscope.
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1. Introduction

Cells can sense mechanical force through which differentiation, development and migration are regulated [1]. Mechanical force works on the cells through the process named mechanotransduction that responsible for conversion of mechanical force into an electrochemical response. It also drives many physiological processes, including touch and pain sensation, hearing, and blood pressure regulation.

One set of biological tools responsible for mechanotransduction is the ion channels called mechanosensitive ion channels (MSCs). Among the variety of MSCs in different systems, one of the most remarkable cation-selective MSC named piezo1 that can respond directly to mechanical force [2]. Tremendous progress has been made in understanding the function and gating mechanisms of this protein since its discovery. As reported, piezo1 has broad roles in multiple physiological processes, including sensing shear stress of blood flow for proper blood vessel development [3], regulating red blood cell function [4] and controlling cell migration and differentiation [5]. Beside the function of piezo1, the gating mechanisms and three-dimensional (3D) structures involving the exact stoichiometry, topology, architecture and functional domains also have been revealed [6].

Despite looking at gating in response to external stimulus, recent studies showed that the function of piezo1 depend on its subcellular localization and dynamics which could be altered by external stimulus [7]. However, little is known about the mechanisms of piezo1 responding to external stimulus, regarding the subcellular localization and dynamics. One of the hypothesis is that the regulations involve the interplay between cytoskeleton and cell membrane triggered by calcium influx. The downstream regulation of the subcellular localization and dynamics in 3D of piezo1 could be rather fast which require a high spatiotemporal imaging tool to solve the out-of-focus problem when tracking the intracellular protein.

To date, in this present study, we utilized a multi-color light-sheet microscopy [8] to multiplex and visualize the previously characterized piezo1-GFP fusion protein and the cellular compartments such as cell membrane, cytoskeleton, to locate the subcellular piezo1 and reveal their interaction. We utilized the light-sheet microscope for imaging single fluorescent proteins in 3D living mammalian cells and provides confocal like spatial resolution with unexceed time resolution and low photo-toxicity, capable for investigating the mechanisms of the regulation of piezo1 to the external stimulus. The GFP fused piezo1 was expressed on HEK 293T cells. After 24 hours, its expression and transportation of piezo1 from its origin to the membrane as well as the movement on the membrane were visualized longitudinally. The piezo1 localization and transportation dynamics in HEK 293T cell with different cell density and osmatic pressure were quantified. Yoda1 which is a piezo1 specific agonist was utilized as a reference stimulus. Real-time and long-term effects of crowding and stretching on the piezo1 localization and dynamics in HEK 293T cells were visualized and characterized by light-sheet microscopy.

2. Results

First of all, we customized a light-sheet microscopy as shown in Fig. 1A. Briefly, the excitation light from the fiber was collimated and shaped by cylindrical lens and single slit and was confined to a Bessel like light sheet with only a few hundred nanometers in thickness along the focal plane, thus almost every photon emitted from the specimen can be captured and contribute to the final image. Therefore, the microscopy is powerful in its low rate of photo toxicity and imaging speed and capable for intracellular molecular tracking. Then, we utilized this system for visualizing the piezo1-GFP fusion protein. The fluorescence profile along the yellow line as shown in Fig. 1B indicates that this system can resolve the protein or protein clusters with around 300 nm resolution.

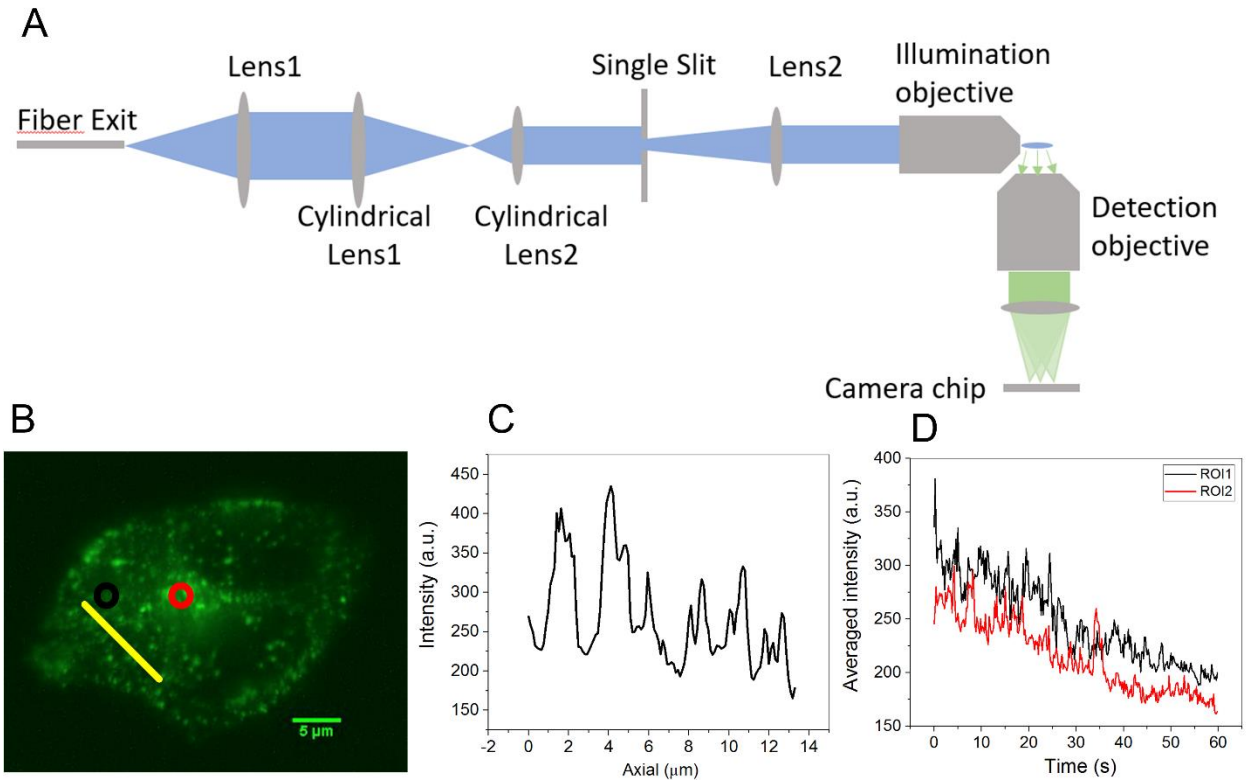

Figure.1. Visualization of GFP fused piezo1 proteins by light-sheet microscopy. A) Scheme of customized light sheet microscopy with subcellular resolution (not drawn to scale); B) Imaging of EGFP-fused piezo1. The green dots indicate the fluorescence of GFP fused piezo1. C) Fluorescence profile along the yellow line in Fig. 1B. D) Fluorescence photobleaching during the experiment.

The result shows that the localization and dynamics of piezo1 in cells can be visualized with unparalleled high spatiotemporal resolution (up to 500 frame/s) (Fig.1B). The piezo1 protein cluster mainly localized on the cell membrane while some of them are located in cytoplasm, which were comparable to the results [7]. The average intensity in ROI1 (circled in black) and ROI2 (circled in red) as a function of the entire monitoring time is shown in Fig.1D. The photobleaching effects is relatively low (~40%) in such high sampling and high power illumination condition.

In addition, we multiplex the cytoskeleton and piezo1. As shown in Fig. 2. As In Fig. 1, the piezo1 is sparsely localized on cell membrane and cytoplasm. Most of piezo1 protein was in cytoplasm and were undergoing highly dynamics which is diffusion like before the activation. The cytoskeleton was stained by SiR800 which is fluorescent upon binding to cytoskeleton. It is shown that cytoskeleton were quite stable and there is not observable interaction between piezo1 and cytoskeleton in this condition. To mimic the regulation of piezo1 and the interaction between piezo1 and cytoskeleton upon piezo1 activation, we utilized Yoda1 which is known as the piezo1 agonist for the artificial piezo1 activation. The Yoda1 drive piezo1 moving outward to the membrane. In the mean time, the actin shrinks and deformed (Fig.2).

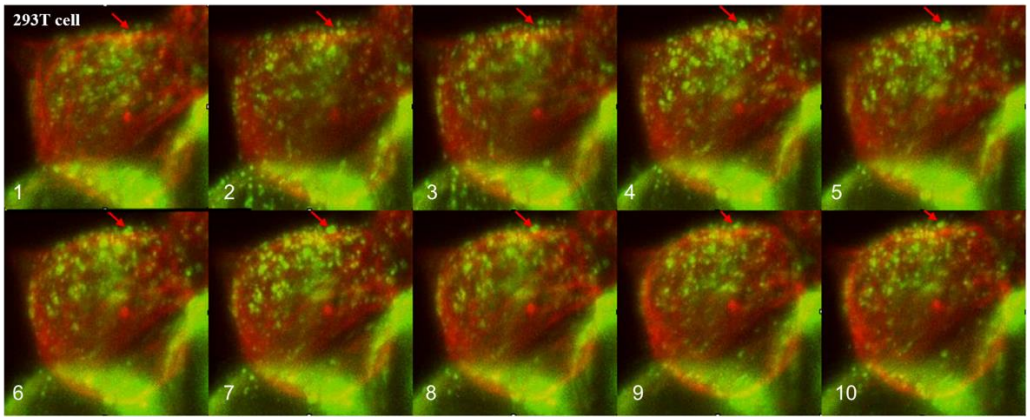

Figure. 2. Piezo1 responses to the Yoda1. The picture shows a living 293T cell transfected with GFP fused piezo1 protein (green) and staining with the cytoskeleton (red). The red arrow indicated the change of a cluster of piezo1 through the whole activation process with 0.1-s intervals.

3. Conclusion

The preliminary data shows that the GFP fused piezo1 protein cluster in 293T cells can be visualized and tracked by the customized light sheet microscopy. The cytoskeleton and piezo1 dynamic change triggered by Yoda1 can be monitored. The 3D dynamic change of protein will be quantified and analysis by using 'Diatrack' software. The effects of external stimulus like osmotic pressure on the localization and dynamics of piezo1 in different cells and the detailed mechanisms will be investigated.

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5. References

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