Long Time Single Molecule Tracking on Receptor Molecules in the Plasma Membrane to Understand Signal Transduction.

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Myriad of external stimuli are sensed by transmembrane receptors in the plasma membrane and processed by the cognate downstream molecules. Receptors and downstream molecules form signaling complexes on the plasma membrane upon signal induction. Thus, formation of signaling complexes should be important for signal transduction in addition to its selectivity, efficiency, spatial and temporal specificity, etc. The signaling complex formation should be detected through analysis of the receptor diffusion motility in the plasma membrane, and therefore trajectories of the receptor diffusion include spatiotemporal information on signaling complex formation. To understand the mechanisms of signal transduction, long time single molecule tracking of receptors and downstream molecules are ideal approach. However, duration of single molecule tracking is limited by photobleaching of fluorescence molecule tagged to the target receptor or downstream molecules. In this study, we used extremely stable fluorescence dye to track motility of platelet derived growth factor receptor (PDGFR) and Akt for consecutive several minutes under low oxygen concentration atmosphere. Based on the results, we discuss the signal transduction mechanism of the PEGFR-Akt signaling system

[Experimental] We established enhanced green fluorescent protein (EGFP)-tagged Akt (Akt-EGFP) and SNAP-tagged PDGFR (SNAP-PEGFR). Akt-EGFP and SNAP-PDGFR were expressed in serum-starved HeLa cells. Twenty four hours after the transfection, Setau647-BG, an extremely stable fluorescence dye was added to the culture medium to stain SNAP-PDGFR, and then the cells were stimulated by PDGF. Total internal reflection fluorescence (TIRF) microscopy observation was performed through from immediately before the PDGF stimulation to 5 min after the stimulation. Then the diffusion motions of PDGFR and Akt were analyzed.

[Results] In the TIRF-based single molecule imaging of Akt-EGFP and SNAP(Setau647)-PDGFR in living Hela cells, fluorescent spots of EGFP and Setau647 that were mainly diffusing on the plasma membrane were observed. The diffusion motility of PDGFR has at least two modes, fast and slow, suggesting they are in different complex-formation states. The detected numbers of Akt-EGFP in 10 sec movies were 632 and 1,259 before and after PDGF stimulation, respectively. We analyzed the residency time of the Akt-EGFP spots on the plasma membrane, and calculated apparent dissociation rate constants kd by fitting the residency time distributions with a single-exponential function. The estimated kd value was 17 s-1 before the stimulation, while was 6.2 s-1 at 5 min. after the stimulation.

[Discussion] Based on the single molecule tracking results, we here propose a new model on signal transduction through Akt. First, receptor molecules in slow diffusion phase form clusters on the plasma membrane upon stimulation. The cluster provides a scaffold of signal transduction; signaling molecules including PIP_3, Akt, and specific downstream molecules are recruited to the clusters. This model explains the mechanism of extension of Akt residency time and selective signal output pattern depend on input signal and is consistent with Akt function as a signaling node. Further discussion will be held in this presentation based on detail analysis of PDGFR motility revealed through long time single molecule observation.