OS02-3 Modulation of Growth Factor Receptors in Membrane Microdomains

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Membrane lateral heterogeneity is accepted as a requirement for the function of biological membranes, and the notion of "raft/microdomain" gives specificity to this concept. Recently, fluorescence-based techniques such as fluorescence recovery after photobleaching (FRAP), single particle tracking (SPT) and fluorescence correlation spectroscopy (FCS) were promising for application to the dynamics of membrane molecules in microdomain. Our previous study has proven a mechanism of insulin resistance, at least in part, in which the dissociation of the Insulin receptor (IR)–caveolin-1 (Cav1) complex is caused by the interaction of IRβ subunit and ganglioside GM3 cluster as glycolipid enriched membrane microdomain by live cell studies using FRAP and SPT techniques. We hope to demonstrate that an alteration of lipid component in microdomain affects lateral diffusion of membrane receptor. Therefore, we established experimental system in which monitored the membrane organization of receptors by analyzing its lateral diffusion parameters in the plasma membrane of living cells using FRAP, SPT. In this study, measurement of the lateral diffusion of IR was performed by fitting analysis to fluorescence recovery curves and trace analysis to individual fluorescent spots, which provided diffusion coefficient (D). It shows us how fast IR molecules are diffusing at the change of membrane environment such as before and after stimulation by cholesterol depression. Now, we established the method which determined diffusion constant of lateral movement of IR-sEGFP or EGFR-mCherry in CHO-K1 cells using these techniques. We will utilize these techniques for the lateral diffusion analysis of membrane receptor in another assay conditions, such as treatment of glycosphingolipid (GSL) inhibitor, use of GSL deficient cells or of pathologic samples.