FEEDBACK INHIBITION OF EGFR DIMERIZATION AS NEGATIVE REGULATOR OF SIGNALING PATHWAY REVEALED BY RASTER IMAGE CLRRELATION MICROSCOPY (RICS)

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Epidermal growth factor (EGF) signaling participates in various cellular aspects including growth, differentiation, survival, and proliferation. The strength and duration of the signal are strictly controlled, and aberrant regulation of the signal causes severe problems such as carcinogenesis. Since the dimerization is critical for the activation EGF receptor (EGFR), modulation of the dimer formation might underlie the mechanism of the regulation of the EGF signaling, but it remains to be explored.

In this study, we expressed EGFR and downstream signaling molecules labeled with fluorescent proteins in CHO-K1 cells and analyzed molecular dynamics of these molecules by raster image correlation spectroscopy (RICS). Upon the EGF addition, the diffusion coefficient (D) of EGFR reduced from $\approx 0.25 \mu m^2/s$ to $\approx 0.11 \mu m^2/s$ in around 2 min. The Number and Brightness analysis revealed that the reduction in D was due to the dimer formation. Following to the initial dimer formation, EGFR repetitively and synchronously monomerized and dimerized with the average repetition rate of 2.5 min. This implies a presence of unreported feedback mechanism that regulates the dimerization state of EGFR.

It has been known that phospholipase Cγ (PLCγ) / protein kinase C (PKC) pathway plays a role in the regulation of the EGF signaling. Cross correlation RICS showed that the PLCγ interacted with EGFR through three phosphotyrosine residues (Y992/1048/1173) of EGFR. We performed mutational analyses and revealed that the PLCγ binding to EGFR play a crucial role for the repetitive monomerization of EGFR. Furthermore, the monomerization relied on PKC and protein kinase D (PKD) while being independent of Ca$^{2+}$ signaling. Phosphorylation of two threonines of EGFR at the juxtamembrane region (T654/669) by PKC and/or PKD was responsible for the monomerization of EGFR. Furthermore, the activity of extracellular signal-regulated kinase (ERK), a downstream signal of EGFR, correlated with the dimerization state of the receptor. We concluded that the monomerization of EGFR via PLCγ / PKC / PKD pathway served as a feedback signaling suppressor.