IBS Center for Molecular Spectroscopy and Dynamics

Seminar

- **SPEAKER**
  Prof. Do-Hyeon Kim (POSTECH)

- **TITLE**
  Direct Visualization of Single-molecule EGFR Activation Process Mediated by Innerleaflet Cholesterol in the Plasma Membrane

- **ABSTRACT**
  Epidermal growth factor receptor (EGFR) controls cell proliferation, growth, motility, and differentiation. The activation mechanism of EGFR has been vastly investigated over past decades as the abnormal activity of EGFR is directly associated with various cancers. Although the activation model of EGFR involving its hydrophilic extracellular and intracellular domains is well established, the mechanism involving the hydrophobic transmembrane domain (TMD) is still poorly understood due to the lack of tools to study lipid-protein interactions in vivo in the plasma membrane where its unique environment significantly contribute to biochemical reactions.

  Previously, we developed a co-immunoimmobilization (Co-II) method that enables to visualize directly the dynamic interactions of single-molecule proteins in living cells. The primary idea of Co-II is intuitive: if bait proteins on the membrane of living cells are immobilized, a prey protein must be immobilized together whenever the interaction occurs. This rapid moment of co-immobilization is captured by single-protein tracking utilizing super-resolution microscopy.

  Here, we visualized single-molecule cholesterol and EGFR interactions in the plasma membrane of living cells by expanding the concept of Co-II to lipid molecules. We found that only the active state of dimeric EGFR physically interacts with cholesterol in the plasma membrane, which seemed contradict to the classical lipid raft model explaining that inactive EGFR resides in the cholesterol-rich rigid domain of the plasma membrane. This contradiction was reconciled by characterizing that free cholesterol in the innerleaflet of the plasma membrane, independent of lipid rafts, participates in the cholesterol-EGFR interaction detected by Co-II.

  Surprisingly, this new type of cholesterol interaction with EGFR was indispensable for EGFR activation by ligands. We revealed that innerleaflet cholesterol serves a critical role in inducing EGF-induced active N-term TMD dimer conformation by locking a

cholesterol molecule inside the cavity produced by TMD and juxtamembrane domains through hydrophobic I640 and L642 residues. This finding alters a paradigm on the current EGFR activation model: innerleaflet cholesterol acts as “a molecular checkpoint” for EGFR activation. We further showed that depletion of innerleaflet cholesterol by ABCA1 and PTCH1b strongly inhibits the hyperactivity of EGFRvIII mutant frequently observed in head and neck squamous cell carcinoma and glioblastoma, implicating the strong relationship between cancer and cholesterol metabolism.

Receptors play pivotal roles in communication between the inside and the outside of cells across the plasma membranes. Various lipids in the plasma membrane have long been considered to influence receptor functions, yet lipid-protein interactions are barely studied due to the lack of tools. Single-molecule Co-II provides an unprecedented tool to open a door to explore lipid-protein interactions in vivo under fluorescence nanoscopy as we demonstrated here by the case of cholesterol and EGFR.

- **DATE AND VENUE**
  December 7, 2021 (Tuesday, 13:00 - 14:00)
  Seminar Room B (119), KU R&D Center

- **LANGUAGE**
  Korean

- **INVITED BY**
  Director Minhaeng Cho