Measuring EGFR dynamics, dimerization and conformation on live cells

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THE Epidermal Growth Factor Receptor (EGFR) is a member of the ErbB family of membrane receptor tyrosine kinases that drive cell growth and survival, with roles in normal development and disease pathogenesis. It is generally accepted that ligand binding to these transmembrane proteins leads to conformational changes, receptor homo- and hetero-oligomerization, kinase activation, and the transphosphorylation of multiple cytoplasmic tail tyrosines. A wealth of structural data supports a model of EGFR signal initiation through the formation of back-to-back homodimers. However, ligand-occupancy status, dimer lifetimes and structure of the receptor remain to be defined on living cells.

We have used multi-color single quantum dot tracking, super-resolution microscopy and FRET-FLIM imaging to determine protein interactions and receptor structure for wild type EGFR and two oncogenic receptor mutants found in non-small cell lung cancer (NSCLC). Our previous work has shown that, during physiological EGFR signaling, stimulation by ligand induces stable dimerization of spatially co-confined and ligand-bound receptors. However, it is unknown whether the constitutive activity of NSCLC mutants arises from changes in oligomeric state within the plasma membrane or is simply due to increased catalytic potency resulting from the mutations in the kinase domain. Using two-color single quantum dot tracking, we show that the NSCLC mutants form stable dimers - independent of ligand. Increased dimerization events are also detected using two-color super-resolution microscopy and localization-based cross-correlation analysis. Finally, FRET measurements of receptor extracellular domain conformation on live cells reveals that the unliganded mutants have an extended structure similar to the ligand-bound wild type receptor. Collectively, these data suggest that the constitutive, unregulated activity of NSCLC mutants is a result of increased dimer affinity and inside-out signaling induced by mutations within the kinase domain.