Integrating Multiplex Single-Molecule Pull-Down (SiMPull) Data and Computational Modeling to Understand EGFR Signaling

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The Epidermal Growth Factor Receptor (EGFR) plays an important role in both physiological and cancer-related processes. To study the factors that influence EGFR phosphorylation, we have coupled single-molecule microscopy experiments with rule-based modeling of EGFR signaling. We have made technical improvements over the previously described Single-Molecule Pull-down (SiMPull) assay to facilitate direct detection of the phosphorylation state of thousands of individual receptors. We monitored the phosphorylation of EGFR-GFP expressed in CHO cells. By counting the number of GFP molecules colocalized with a red-emitting fluorescent antibody, the fraction of receptors phosphorylated at a specific tyrosine residue was determined. We found that only a subpopulation of EGFR become phosphorylated under what is considered maximal activation conditions and that the extent of phosphorylation varies by tyrosine residue. Three-color imaging of EGFR-GFP with antibodies directed to two distinct phospho-sites revealed that multi-site phosphorylation frequently occurs.

To better understand the implications of these results, we created a computational model of EGFR signaling. In our model, a phosphorylated site cannot be dephosphorylated if it is bound by one of its protein binding partners, such as the adaptor protein Grb2. Our model predicted that an increase in the abundance of Grb2 would result in a higher percentage of receptors phosphorylated at sites to which Grb2 binds. In agreement with this prediction, overexpression of Grb2 caused a dramatic increase in the phosphorylation levels of a Grb2-binding site in EGFR (Y1068), but not in a site which Grb2 does not bind (Y1173). These results demonstrate the importance of receptor:adaptor protein ratios in modulating receptor phosphorylation patterns. Since protein abundance varies across cell types and is often altered in cancer, we are currently extending these studies to cancer cells lines with markedly different EGFR:Grb2 ratios.

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