Spatio-Mechanical Inputs Alter ErbB Receptor Functions

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Short Abstract —

Cell survival depends on the accurate recognition and response of surface receptors to their immediate chemical environment. The homo- and hetero-association of membrane receptors at multiple length scales is believed to be a key step in the initiation of biochemical signals. For example, the receptor tyrosine kinase ErbB2, which is overexpressed in 20-30% of breast cancers, can be activated by clustering with other ErbB family receptor proteins or through overexpression-driven oligomerization. We investigate the existence and importance of large scale (from 10 nm to 1 micron in scale) ErbB associations in living cells using nanopatterned synthetic interfaces. A suite of bottom-up and top-down nanofabrication techniques are used to tailor supported lipid membranes that are decorated with specific ligands. Signaling outputs are measured using a range of bioanalytical tools in order to quantify cellular response. The results of these investigations will be presented in the context of the ErbB signaling pathways in cancer cell lines known to overexpress the receptor.

***Keywords* — Single cell manipulation, mechano transduction, supported lipid membranes, signaling, ErbB receptor, EGFR, nanopatterned surface.**

I. Introduction

A new and emerging paradigm in cell-to-cell communication is the concept that the large-scale spatial organization of signaling molecules can influence their biochemical functions. Signaling assemblies consist of tens, hundreds, thousands and perhaps tens of thousands of signaling molecules where the properties of the assembly can regulate the individual properties of its molecular components. Hierarchical organization of signaling components from the molecular to micrometer length scales feed into signaling pathways to regulate collective cell signaling outcomes (1). For example, T-cell receptor activation has been found to be location dependent in the immunological synapse (2, 3), and the activity of the EphA2 receptor tyrosine kinase can be modulated based on the cluster size and transport of the ligand-receptor complexes (4). This poses as a challenge for the quantitative study and ultimate understanding of cellular signaling. Consequently, new tools are required to deconstruct the precise interplay between spatial and mechanical components in signaling pathways.

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II. Receptor Manipulation – Approach and Results

The ErbB receptors are receptor tyrosine kinases, that when deregulated become implicated in a variety of human carcinomas. The formation of ErbB1 receptor oligomers at multiple length scales is a key step in the initiation and propagation of their biochemical signals (1). For example, single molecule tracking experiments provide insight into the formation of receptor nanoscale assemblies (5). Global mechanistic understanding requires the ability to introduce mutations in spatial organization. To address this question, we are applying the spatial mutation strategy to deconstruct the ErbB1 receptor pathway (4). In this strategy, ligands are anchored to a synthetic supported membrane that is fluid in two dimensions. Nanoscale corrals patterned in the solid support guide the organization of the receptors on the surface of living cells. This allows external control of receptor organization. The ErbB1 receptor is found to be spatially reconfigured in the hybrid cell – synthetic membrane interface. Signaling outcomes were measured as a function of the dimensions of nanopatterned substrates by using single cell microscopy approaches. We present quantitative data indicating that nanopatterned barriers act to constrict receptor motions and this, in turn, modulates receptor signaling. These results provide for one mechanism in which receptor function can be fine-tuned without the need to introduce genetic mutations or post-translation modification in its structure.

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