

Exploring in vivo cholesterol-mediated interactions between activated egf receptors in plasma membrane with single-molecule optical tracking

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ABSTRACT Background: The first step in many cellular signaling processes occurs at various types of receptors in the plasma membrane. Membrane cholesterol can alter these signaling pathways of living cells. However, the process in which the interaction of activated receptors is modulated by cholesterol remains unclear.

Methods: In this study, we measured single-molecule optical trajectories of epidermal growth factor receptors moving in the plasma membranes of two cancerous cell lines and one normal endothelial cell line. A stochastic model was developed and applied to identify critical information from single-molecule trajectories.

INDEX TERMS Autotrophs, Cytokinesis, Heterozygous., Unicellular microorganism, A coordinating organ of human body, Juicy fluid within flowers

1. Background

Receptor proteins are ubiquitous in the plasma membrane of mammalian cells, which transduce information about cellular environment to intracellular signaling networks [1]–[3]. There are approximately 1352 receptor proteins coded by human genome [4]. The flow of information through those receptors is critically shaped by receptor interactions. The diverse cellular processes regulated by such receptor proteins include cell growth and division, differentiation, migration, and apoptosis [5]. Receptor signaling dysregulation is attributed to the pathogenesis of several diseases [6], [7]. Therefore, understanding the interactions, molecular processes and relevant structures of such signaling receptors is imperative.

Human receptor proteins, including the epidermal growth factor receptor (EGFR), share a common molecular architecture, consisting of an extracellular binding domain, a single transmembrane helix, a flexible juxtamembrane segment, and an intracellular tyrosine kinase domain [8]. Ligand binding to the extracellular domains induces conformational reorganization that promotes receptor dimerization, leading to the activation of the intracellular tyrosine kinase domain. However, conclusions about the mechanisms of ligand-induced dynamic

recruitment and dimerization of membrane-associated receptors remains controversial [9]. Conventional steady-state ensemble approaches cannot be used to address the stochastic nature of activated receptors that encounter each other in a highly heterogeneous and fluidic plasma membrane. Recent advancements in single-molecule fluorescent imaging and tracking have provided further insights into the behavior of EGFR in vivo [10]–[12]. For example, Lidke et al. devised a two-color quantum-dot tracking method to visualize the state-dependent dimerization processes of human EGFR in living cells [13]. A hidden Markov model was used to extract the kinetic parameters of the underlying free, co-confined, and dimerized states.

Researchers have increasingly determined that lipid domains rich in glycosphingolipids and cholesterol can facilitate signaling receptors to form a dimer [13]–[15]. Because the recruitment mechanism and dynamic clustering of receptors in the hierarchical structure of plasma membranes are not clearly understood, the existence and functionality of lipid nanoscale domains on EGFR dimerization remains controversial. A growing body of research indicates that membrane cholesterol can influence the organization, stability, and function of membrane proteins including re-

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