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Correlative Super-resolution Fluorescence and Electron Microscopies Unravel the Nanocluster Formation of Ras proteins on the Cell Membrane

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Abstract

Physical and functional partitioning of the biological membrane have been implicated in regulating heterotypic and homotypic interactions of proteins on the membrane. Among others, human Ras small GTPases are prototypical examples of membrane proteins that have been shown to preferentially compartmentalize on the plasma membrane. All three Ras isoforms, namely H-, N-, and K-Ras exhibit nanoclusters at different locations within membrane, which is primarily dictated by their isoform-specific membrane-targeting motifs. This spatial preference among the isoforms could explain why Ras proteins have non-redundant biological functions and distinct mutational spectra in human cancers despite the high homology in their functional (globular) domains. Thus, understanding how Ras is partitioned into nanoclusters on the plasma membrane is critical for devising mutant Ras-targeted cancer therapy. Using quantitative super-resolution fluorescence microscopy (SRM), we observed that two mutant Ras forms, K-Ras and H-Ras, can form either dimers or higher clusters on the cell membrane when expressed at near-endogenous expression levels. Furthermore, the correlative SRM and scanning electron microscopy revealed that both H-Ras and K-Ras dimers/nanoclusters are independently associated with distinct subsets of plasma membrane ultrastructures, such as cortical cytoskeleton, clathrin-coat pits, and caveolae. Additionally, we found that the membrane clustering properties are dominated by the C-terminal membrane targeting motifs of both Ras isoforms. Together, these results expand our understanding of structural and functional properties of Ras nanoclusters on the cell membrane and offer guidance to future studies defining membrane structures associated with Ras.