EGFR CLUSTERING AWAY FROM CHOLESTEROL- OR SPHINGOLIPID-ENRICHED MICRODOMAINS REVEALED BY PALM/DSTORM

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Lipids in biomembranes such as cholesterol and sphingomyelin form raft-like microdomains. These microdomains are thought to serve as platforms for signal transduction and molecular trafficking, but it is difficult to elucidate their detailed structure since their size is smaller than the resolution of light microscopy. We employed superresolution microscopy to circumvent this limitation. To this end, we designed dedicated probes for cholesterol- and sphingomyelin-enriched microdomains (Figure 1) [1]. PALM imaging revealed two types of cholesterol-enriched microdomains, line-shaped with widths of around 150 nm and round-shaped with the average radius of 118 nm. All sphingomyelin-enriched microdomains were round-shaped with an average radius of 124 nm. Both the cholesterol- and sphingomyelin-enriched microdomains vanished by the depletion of cholesterol. The sphingomyelin-enriched microdomains also vanished by the depletion of sphingomyelin whereas the cholesterol-enriched microdomains were unaffected. We conclude that cholesterol- and sphingomyelin-enriched domains occupy different regions on the plasma membrane. EGF receptors clustered on the plasma membrane with no apparent change upon short stimulation with EGF. The clusters of EGF receptors were on different regions from cholesterol- or sphingomyelin-enriched microdomains and unaffected by depletion of cholesterol and sphingomyelin. The clusters were gathered and endocytosed upon prolonged stimulation with EGF. The endocytosis required membrane cholesterol but not sphingomyelin.