Extracellular matrix (ECM) stiffness regulates cell differentiation, survival, and migration. Our previous study has shown that the interaction of the focal adhesion protein vinculin with vinexin α plays a critical role in sensing ECM stiffness and regulating stiffness-dependent cell migration. However, the mechanism how vinculin–vinexin α interaction affects stiffness-dependent cell migration is unclear. Lipid rafts are membrane microdomains that are known to affect ECM-induced signals and cell behaviors. Here, we show that vinculin and vinexin α can localize to lipid rafts. Cell-ECM adhesion, intracellular tension, and a rigid ECM promote vinculin distribution to lipid rafts. The disruption of lipid rafts with Methyl-β-cyclodextrin impaired the ECM stiffness-mediated regulation of vinculin behavior and rapid cell migration on rigid ECM. These results indicate that lipid rafts play an important role in ECM-stiffness regulation of cell migration via vinculin.

Vinculin is in non-raft on soft ECM. Rigid ECM promotes vinculin binding to vinexin α and PIP_2, leading to distribution to raft and enhanced migration