Structural Organization of E-Cadherin and its Interactome Probed by Proteomics and Super-resolution Microscopy

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Abstract

E-cadherin-mediated cell-cell adhesion and signaling plays an essential role in development and maintenance of healthy epithelial tissues. Adhesiveness is mediated by its extracellular domains and is regulated by a network of adaptors and enzymes associated with its cytoplasmic tail. We used proximity biotinylation and quantitative proteomics to identify 612 proteins in the vicinity of E-cadherin's cytoplasmic tail. We used a structure-informed database of protein-protein interactions to construct a comprehensive E-cadherin interactome, containing 89 known and 346 previously uncharacteized proteins. Importantly, we found that most protein interactions with E-cadherin were not disrupted by calcium chelation or myosin inhibition, suggesting the majority of the E-cadhesome is independent of cell-cell adhesion and actomyosin contractility. Next, we used super-resolution microscopy to elucidate the nanoscale architecture of adherens junctions at 30nm resolution, and evaluated the contributions of the extracellular domain, cytoplasmic interactions, and filamentous (F)-actin. We found junctions to be composed of discrete clusters, the majority of them with 3-10 E-cadherin receptors at a density of 36 molecules/$(100nm)^2$, which is 10-fold lower than the crystal packing density. Surprisingly, such clusters, which are also found along the lateral junction, form independently of trans- or cis-interactions. In agreement with this observation, differential labeling of E-cadherin in neighboring cells revealed that while the proportion of adhesive clusters in apical junctions was high, only a subset of lateral clusters were adhesive. Strikingly, we discovered that the cytoplasmic tail of E-cadherin limited cluster size, and determined this to be a result of its interaction with F-actin. Dual-color super-resolution imaging of E-cadherin with F-actin revealed a mutually exclusive localization, in which F-actin surrounded E-cadherin clusters. In conclusion, our findings suggest that the basic unit of E-cadherin adhesion is a cluster composed of a small number of E-cadherin receptors, corralled by F-actin, which forms numerous interactions with cytoplasmic proteins independently of trans-interactions.