Abstract

Epithelial Cell Adhesion Molecule (EpCAM) is a type I transmembrane protein, which plays a key role in cell proliferation, differentiation and adhesion. It is expressed in epithelial and stem cells. Due to its frequent overexpression in various carcinomas it is also recognized as a promising tumor marker. EpCAM is directly involved in cell-cell adhesion and signaling. Homo-oligomerization has an important role in both those processes. Through homophilic interactions, EpCAM forms calcium independent cell-cell contacts and its oligomeric state regulates proteolytic cleavages during signaling. Mechanism and regulation of oligomerization are yet to be described in detail. It is generally accepted that EpCAM forms *cis*-dimers and *trans*-tetramers, which arise from interaction of two *cis*-dimers on opposing cells. Here we present a detailed structural and biochemical analysis of EpCAM oligomerization, employing four complementary experimental approaches (SAXS, XL-MS, BAA and FLIM-FRET). Results of SAXS, XL-MS and BAA clearly indicate EpCAM forms *cis*-dimers, but not *trans*-tetramers in solution. We were not able to detect any biologically relevant interactions between EpCAM's extracellular domains, which are a prerequisite for formation of homophilic cell-cell contacts. Similar conclusions can be made based of our FLIM-FRET analysis in cells. While our results present the first experimental conformation of *cis*-dimerization *in vivo*, we haven't been able to detect any inter-cellular interaction between EpCAM molecules, localized at the areas of cell-cell contacts. Our work provides clear evidence that EpCAM is fact not a homophilic cell-cell adhesion molecule, which calls for a significant revision of its actual role in cells. Our findings will help explain some seemingly contradicting reports regarding EpCAM's function and thus greatly contribute to our understanding of processes involved in epithelial development and organization, as well as carcinogenesis.

Keywords EpCAM, cell-cell adhesion, oligomerization