N-terminal domain of EpCAM is involved in proteolysis-associated signaling.

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EpCAM is a homodimeric transmembrane glycoprotein associated with enchanced cell proliferation and cancer through regulated proteolysis-associated signaling. This involves a series of cleavages carried out by three membrane-bound proteases, TACE (TNFα converting enzyme), BACE1 (β-secretase 1) and γ-secretase, and results in the release of the EpCAMs intracellular part, a crucial component for the formation of the transcription complex responsible for increased mitogen synthesis. The signaling is initiated by the cleavage of the extracellular part by either TACE or BACE1, however the details of molecular events leading to cleavege at sites buried in the dimerization surface of EpCAM remain unknown.

One possible factor regulating the accessibility of the cleavage sites is interaction of EpCAM with other proteins which can lead to the exposure of the cleavage sites, i.e. by causing the dimer dissociation. In order to test this hypothesis we performed interaction interface mapping by mutating surface-exposed residues of EpCAM's extracellular part (EpEX). A set of 28 of such mutants was studied *in vivo* and the extent of the cleavage was assessed with the quantitive western blot. The analysis revealed three distinct surface patches with the same cleavage pattern compared to the wild type, however the extent of the cleavage at certain sites was substantialy different. These surface regions represent possible interaction sites with proteins affecting the cleavage and are located mostly on the membrane-distal N-terminal domain. Those interaction partners will be identified using a proximity ligation assay involving promiscuous biotin ligases BioID2 and/or TurboID fused to the extracellular part of EpCAM and will enable us to determine which interaction partners bind to which surface patch and hopefully reveal their underlying effect on the signaling initiation.