EpCAM interactions as determinants of signaling via regulated proteolysis

Abstract

EpCAM is a dimeric type I transmembrane protein that plays a crucial role in normal embryonic development and the maintenance of intestinal epithelial integrity in developing and adult organisms. Its expression is frequently upregulated in various carcinomas, making it an important tumor marker and therapeutic target. Initial experiments suggested that EpCAM functions as a cell adhesion molecule; however, subsequent studies have refuted this notion, shifting the focus of research to its signaling role. EpCAM is involved in several signaling pathways, the most important of them being the RIP-activated Wnt/β-catenin signaling pathway, responsible 1ort hi majority of EpCAM oncogenic effects, such as increased proliferation, migration, and invasion. RIP is initiated by the EpCAM ectodomain (EpEX) shedding by proteases TACE or BACE1, followed by intramembrane proteolytic processing by γ-secretase and release of the EpCAM intracellular domain (EpICD), which is involved in nuclear signaling and induction of pathway-related protein expression. EpEX shedding is a critical step in initiating RIP, yet the factors influencing this process are not well understood. The aim of this doctoral work was to investigate two factors, possibly involved in EpEX shedding: the oligomeric state of EpCAM and its potential interaction partners.

EpCAM exists in a dimeric form, with the main dimerization surface formed between EpEX subunits, which can also dimerize independently without the rest od EpCAM. The cleavage sites of TACE and BACE1, responsible for EpEX cleavage, are located within its dimerization interface, rendering them inaccessible to proteases in the dimeric state of EpCAM. For successful cleavage, the EpCAM dimer must dissociate into monomeric subunits which was demonstrated by the cleavage experiment using isolated monomeric and covalently linked dimeric EpEX mutants with the TACE ectodomain. The cleavage assay revealed that EpEX oligomeric state is crucial for its cleavage, as only the monomeric form of EpEX is susceptible to cleavage by the TACE ectodomain.

In addition to the impact of the oligomeric state of EpCAM on EpEX shedding, we also investigated the possible effect of its potential interaction partners, focusing on the interaction partners of the EpEX itself, which represents the major interaction surface of EpCAM. The experiment was divided into two parts.

In the first part, we identified potential interaction surfaces on the EpEX, and in the second part, we identified its potential interaction partners. Potential interaction surfaces were identified through site-directed screening mutagenesis of amino acid residues on the surface of EpEX, aiming to selectively weaken interactions with potential interaction partners. The effect of reduced interaction on the extent of EpEX shedding was assessed quantitatively by analyzing the shedding extent of EpCAM mutants in the colorectal carcinoma cell line HCT8.

Among the twenty-eight mutants tested, the shedding extent significantly differed from the wild type in six mutants, with an increased shedding extent observed in all of them. The mutated amino acid residues within these six mutation sets represent potential contact surfaces through which proteins diminishing EpEX shedding bind to EpCAM. Notably, four of the six significant mutation sets lie in the N-terminal domain of EpEX, suggesting that the N-terminal domain may represent an important interaction surface.

Cleavage analysis of EpCAM mutants further revealed that cleavages by TACE or BACE1 and matriptase, a protease that cleaves EpEX within the thyroglobulin loop, seem to be mutually exclusive. This finding appears contradictory since matriptase-cleaved EpEX is unable to dimerize, which leads to the destabilization of the EpCAM dimer and exposure of TACE and BACE1 cleavage sites and should have a synergistic effect on shedding. It is currently unclear whether the observed effect is a result of altered EpCAM interaction with proteases directly 2ort it involves other factors such as altered protein trafficking or localization within other membrane domains.

The second part of the experiment involved the identification of potential interaction partners of EpCAM using the biotinylation-based proximity labeling technique utilizing the promiscuous biotin ligase TurboID and mass spectrometry performed in the colorectal carcinoma cell line HCT8. 2ort his purpose, we developed a system for specific labeling of proteins on the cell membrane, based on the indirect binding of TurboID to EpCAM after its synthesis and translocation to the cell membrane. This approach allows for temporally and spatially controlled labeling of EpCAM-proximal proteins on the cell surface. The experiment yielded a set of candidate interaction partners of EpCAM, including membrane proteins that are involved in known EpCAM functions, as well as proteins that open new possibilities for functional research, necessitating further validation studies.

This work provides a solid foundation for further investigations into the factors influencing EpEX shedding and the potential regulation of EpCAM signaling through RIP. It paves the way for a more comprehensive understanding of the role of EpCAM in the cell, particularly regarding its significance in cancer development and progression.

Keywords: EpCAM, signaling, RIP, mutagenesis, interaction surfaces, interaction partners, biotinylation-based proximity labeling, TurboID, mass spectrometry.