Optimized expression and purification of the ectodomain of the disintegrin metalloprotease ADAM17 and the design of a cleavage assay for EpCAM

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K.P. van Midden[I], B. Lenarčič[I], A. Gaber[I]

[I]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Vecna pot 113, Ljubljana, Slovenia

The disintegrin metalloprotease ADAM17, also known as the tumor necrosis factor converting enzyme (TACE), is an essential regulator of several signaling pathways that include development, immunity, inflammation, and cancer progression. This is possible because it cleaves a number of different substrates including cytokines, growth factors, and adhesion molecules. Even though TACE has been extensively studied, molecular mechanisms that govern cleavage of some substrates have yet to be elucidated. One of these substrates is the transmembrane glycoprotein EpCAM, which has been identified as a tumor-associated antigen due to its high expression level in rapidly growing epithelial tumors. *In vivo* studies have shown that TACE cleaves EpCAM at two alpha sites, which are both located in a groove formed by two subunits in a dimer – the native oligomeric state of EpCAM. The cleavage sites are thereby inaccessible to TACE. To better understand the mechanism by which TACE is able to cleave EpCAM we designed various full-length and extracellular constructs of TACE and expressed them in an Sf9 insect cell line. We optimized the expression and purification methods for the construct containing the entire wild-type ectodomain (TACE-EX) and performed the first *in vitro* cleavage of EpCAM. We also designed and tested the experimental procedure to analyze the cleavage of the soluble EpCAM ectodomain. Our optimized expression and purification protocols will enable further studies of *in vitro* cleavage of different TACE substrates and allow us to better understand the underlying mechanisms of EpCAM cleavage.