Abstract

Trop-2 (Trophoblast-specific surface antigen 2) is an epithelial transmembrane glycoprotein expressed at high levels during embriogenesis and malignant transformation. Due to its abundance on carcinoma cells it has been classified as a potential target for tumor therapies, similarly to its homologue EpCAM. The goal of our research was a detailed structural and biochemical characterization of Trop-2, and to illuminate the similarities as well as differences between Trop-2 and EpCAM.

Due to the complexity associated with transmembrane protein research we studied the multidomain extracellular part (composed of N-terminal, thyroglobulin and C-terminal domain) and the cytosolic part of Trop-2 separately. First, we optimized the preparation procedure for recombinant extracellular part. This large fragment of the molecule forms a dimer at physyological pH, however at higher pH values formation of different aggregates was observed. Side-by-side analysis of thermal stability of Trop-2 and EpCAM extracellular parts revealed that the stability of the latter is considerably lower. We ascribe this to the differences between amino acid sequences of the N- and C-terminal domains that show the lowest degree of homology between the two proteins. Next, we showed that the IGF-1 binds to the monomeric as well as to the dimeric form of Trop-2 extracellular part.

We have also prepared crystals of Trop-2 extracellular parts and recorded several sets of diffraction data with the highest resolution of 2.94 Å. Structure of Trop-2 extracellular part was solved by the means of molecular replacement using the structure of EpCAM extracellular part as model. Building of a complete structure was not possible due to undefined electron density for several parts of the molecule, particularly in the region of the N-terminal domain.

Additionally, we performed detailed structural characterisation of the Trop-2 cytosolic part with emphasis on structural changes induced by phosphorylation of Ser^{303}. Initial analysis showed that this part is unstructured under physiological conditions. For further studies we used 2,2,2-trifluroethanol as a co-solvent, which mimics the environment found at protein-protein interaction interfaces. In this manner we were able to determine NMR structure of both non-phoshorylated and phoshorylated form of the cytosolic part. The non-phoshorylated form adopts an L-shaped structure composed of an α-helix and a shorter 3_{10}-helix. Upon phoshorylation considerable structural differences were observed – molecule adopts a curved rod-like structure, and the conformation of a distal proline residue is shifted from *trans* to *cis*.

Our results significantly contribute to the understanding of Trop-2 structure and function, and at the same time provide a solid foundation for further research in terms of detailed charactarization of protein interactions.

Key words:

Trop-2, dimerization, crystallization, thermal stability, phosphorylation