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Summary of doctoral dissertation

"Studies of interactions between human cystatin C and monoclonal antibody HCC3"

Human cystatin C is a small protein (13.3 kDa) that is commonly found in human body fluids where it acts mainly as an inhibitor of cysteine proteinases. In pathological conditions, this protein forms dimers and higher oligomeric forms, which can lead to their retention in various organs. This may lead to the development of an amyloid disease, particularly in patients with a rare mutation of human cystatin C, L68Q. This mutation is the cause of hereditary cystatin C amyloid angiopathy, HCCAA, resulting in deadly cerebral haemorrhages, even in young people.

Many antibodies are known to interact with human cystatin C. Professor Grubb (University of Lund) and colleagues have shown that some of the antibodies tested significantly inhibit formation of hCC dimers/oligomers, while others have little effect on inhibiting the formation of hCC dimers. Mouse monoclonal antibodies, HCC3, obtained by the above-mentioned research team, inhibit the formation of hCC dimers to a significant extent (about 60%). This prompted me to further research on these antibodies and to identify sites of binding of the hCC protein with the antibody: epitope (a protein sequence responsible for a formation of the complex with an antibody) and paratope (an antibody sequence binding to an antigen). Information about the identified epitope and paratope can be extremely important in the design of HCCAA immunotherapy.

Using several various, complementary epitope identification methods: (i) MS-assisted epitope excision and extraction using enzymatic digestion (trypsin, Asp-N endoproteinase, pronase), (ii) affinity chromatography with the pre-identified (on the basis of the digestion results) hCC fragments, (iii) enzyme-linked immunoassays (ELISA) and (iv) hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) I showed that the epitope is

nonlinear and consists of at least two protein fragments. In order to find mAb HCC3 features necessary to inhibit the dimerization of hCC and confirm the mechanism of the protein dimerization, the results of the epitope identification were compared with the results on the interaction sites for two other antibodies interacting with hCC – mAb Cys10, which inhibits dimerization the least (4%) and mAb Cys28, which inhibits dimerization the most (75 %).

As part of my doctoral dissertation, I focused on the characteristics of the HCC3 antibody, determining its mass, subclass membership, qualitative and quantitative oligosaccharide profile and primary structure of the antibody, identifying light chain sequence (212 Aa) in 93%, and heavy chain (439 Aa) in 90% . Using the Kabat's and Chothia's rules for theoretical determination of a paratope (6 complementary determining regions, CDRs), I determined four sequences corresponding to CDR loops – all three light chain loops and one heavy chain loop (due to the incomplete sequence). Experimental results of the paratope identification (HDX-MS and Parexprot methods) confirmed some its fragments, and the affinity chromatography of the synthesized peptides corresponding to the sequences of the three CDRs of the light chain with human cystatin C confirmed the formation of a human cystatin C complex with two CDRs.