Abstract

„Molecular characterization of the HVEM-CD160 complex as a target in immunotherapy”

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The immune checkpoints comprise a group of proteins which can modulate the activity of the immune system through its stimulation or inhibition. Some of these proteins are already well known e.g. CTLA-4 and PD1. However, there also exist such, we know very little about. The cluster of differentiation 160 protein (CD160) is one of the recently discovered immune checkpoints. Its structure and functions were not yet unambiguously described. The CD160 protein occurs i.a. on the surface of T lymphocytes. It interacts with the herpesvirus entry mediator protein (HVEM), which is present on antigen-presenting cells and cancer cells. The formation of CD160–HVEM complex causes the inhibition of T lymphocyte activation, their proliferation and cytokine production. An increasing amount of studies indicates that the CD160–HVEM complex may be a target in the treatment of cancer, virus infections and immune diseases. Numerous studies proof that the complex may take part in many yet to be discovered immune system mechanisms.

The presented thesis focused on the molecular characterization of CD160–HVEM protein complex. The information on the CD160 structure is scarce. Therefore, in the first stage of my studies I decided to verify if the protein occurs as a monomer, dimer or forms higher oligomers in aqueous solution. For this purpose, I used SDS-PAGE electrophoresis, size exclusion chromatography, Ellman’s test and mass spectrometry and established that the protein is a monomer and its amino acid sequence contains one cysteine residue with a free sulfhydryl group. Next, I used molecular modeling methods and the hydrogen-deuterium exchange mass spectrometry and determined the tertiary structure of the CD160 protein. The structure shows that the protein is formed by two β-sheets. One of the sheets contains four β-strands and the other one five β-strands.

The next stage of my research comprised the determination of the fragments of CD160 amino acid sequences involved in the interaction with the HVEM protein and the deamination of CD160–HVEM complex structure. The literature shows that the HVEM protein binds to the CD160 with the CRD1 domain which contains c.a. 40 amino acid residues. Therefore, in my studies I focused on the identification of binding sites in the CD160 protein. To identify the

fragments in the CD160 protein involved in the interaction with HVEM protein, I used the hydrogen-deuterium exchange mass spectrometry. I was able to establish that the following CD160 fragments – CD160 (16-21), CD160 (30-34) and CD160 (76-87), take part in the interaction with HVEM protein. Next, I divided the CD160 sequence into ten fragments and synthetized them. With the use of affinity chromatography and immunoenzymatic tests I determined four peptides (CD160 (13-32), CD160 (25-44), CD160 (61-80), CD160 (73-92)) exhibiting the affinity to the HVEM protein.

Considering the fact that, the formation of CD160–HVEM complex inhibits the activity of immune system I also designed potential inhibitors preventing the binding of the mentioned proteins. I aimed at designing compounds binding to the HVEM protein. For this reason, I decided to verify if the fragments of CD160 sequence I identified inhibit binding of the studied proteins. The CRD1 domain of the HVEM protein, which binds the CD160 protein, is also involved in the interactions with B- and T-lymphocyte attenuator (BTLA) and glycoprotein D (gD). Therefore, I decided to use the amino acid sequences of those proteins during the design of the inhibitors. Next, I designed peptides – fragments of CD160, HVEM and gD (potential inhibitors), and verified their influence on the formation of CD160–HVEM complex with the use of competitive immunoenzymatic tests. The performed studies allowed the identification of two peptides (CD160(13-39)(Cys6-Asp37Cys) oraz gD(1-36)(Lys10Cys-Thr29Cys)) which inhibit the formation of CD160–HVEM complex.