Insights in peptidyl substrate binding to cysteine cathepsins

P-02.3-10

J. Loboda[I], P. Sosnowski[II], L. Tušar[II,III], R. Vidmar[II], M. Vizovišek[II], J. Horvat[IV], G. Kosec[IV], F. Impens[V,VI], H. Demol[VII], B. Turk[II], K. Gevaert[VI,VII], D. Turk[II,III]

[I]PhD student, Ljubljana, Slovenia, [II]Department of Molecular and Structural Biology, Jozef Stefan Institute, Jamova 39, Ljubljana, Slovenia, [III]Centre of excellence CIPKEBIP, Jamova 39, Ljubljana, Slovenia, [IV]Acies Bio d.o.o., Tehnološki park 21, Ljubljana, Slovenia, [V]VIB Center for Medical Biotechnology, A. Baertsoenkaai 3,, Ghent, Belgium, [VI]Department of Biomolecular Medicine, Ghent University, A. Baertsoenkaai 3,, Ghent, Belgium, [VII]VIB Center for Medical Biotechnology, A. Baertsoenkaai 3, Ghent, Belgium

Cysteine cathepsins are lysosomal peptidases involved in numerous physiological and pathological processes, such as protein degradation, protein processing, antigen presentation, cancer and CNS (central nervous system) disorders. We are trying to understand how these proteases select their endogenous substrates. Analysis of proteomic study of the cell lysate, which was enriched with selected cathepsins, suggested representative peptides as a model of protein substrates based on cathepsin's specific cleavages. Using a structural approach, we attempted to validate the peptide model with the crystal structures of active-site mutant human cathepsin V in complex with those peptides. The results showed that binding to cathepsin was affected by primary sequence of the peptides, their terminal modification, crystallization conditions and cathepsin active site mutation. Further on, those same peptides were treated with native cathepsin V, K and L and cleavage sites were identified by HPLC-MS. We showed that peptide binding or cleavage don't always match with the binding and/or cleavage of protein substrate. Hence, peptides are not (always) the model to rely on when studying substrate specificity at least of cathepsins V, K and L, but likely also other endopeptidases.