Establishing cyanobacterial peptide nostatin A as a novel V-ATPase inhibitor in human cells

Ribosomally synthesized and post-translationally modified peptides (RiPPs) possess specific structural and conformational properties that enhance their stability in biological systems and ensure selective bioactivity. This make RiPPs suitable scaffolds for development of peptide based drugs. Our research team has discovered a unique type of RiPP belonging to the proteusin family, which currently includes only a few described chemical structures. Due to its strong anti-proliferative potency and low systemic toxicity, nostatin A is a promising anti-cancer lead. However, to better define its potential use, the knowledge on the exact mechanism and mode of action is essential. Our collaborative long-term project with Centre for Molecular Medicine (CeMM) in Vienna was based on application of omic-based methods and detail characterization of cell-death signalling pathways to uncover nostatin A mode of action. The proteomics and transriptomics analyses has shown mild but significant upregulation of lysosomal degradation machinery and cholesterol metabolism, respectively. Using a fluorescently labelled compound we have further demonstrated that nostatin A localizes into lysosomes. Finally, chemo-proteomic pull-down experiment with bead-linked nostatin A provided convincing evidence that the molecule interacts with V0 subunit of vacuolar ATPase (V-ATPase). These results collectively show that nostatin A inhibits V-ATPase involved in lysosome acidification. Interestingly nostatin A-induced lysosomal impairment resulted in upregulation of down-stream cascades including stress of endoplasmic reticulum, amino-acid deprivation signalling cascade and cholesterol biosynthesis pathway. These data add a relevant piece on information for mode of action of V-ATPase inhibitors in general. As V-ATPase is especially important for marcopinocytosis and metastases formation in specific types of cancer, these findings defines the further direction and nostatin A application.