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Monitoring of granzyme B activity in biological systems using new chemical tools.

Natural Killer Cells and cytotoxic T lymphocytes are effector cells of the innate immune system involved in the elimination processes of pathogens and tumor cells. Enzymes involved in these processes are, among others, granzymes - a group of serine proteases found in the granules of the these cells. Despite many years of intensive research on the granzyme family, their exact function and location remain vague. Facing this problem, this doctoral dissertation focuses on the investigation of the location and activity of granzyme B (GrB) in cell lines with the developed by our group chemical tools containing unique amino acid sequences in their structure.

The first phase of the research included the determination of granzyme B active site specificity profiles. For this purpose, the hybrid combinatorial fluorogenic substrate library (HyCoSuL) technology and defined substrate libraries containing a wide range of unnatural amino acids were applied. This allowed for the precise determination of the best recognized amino acid residues in the S5-S1 pockets of the active site of GrB. The use of a combination of the above two techniques provided the basis for the design and synthesis of specific tools for study activity of granzyme B: fluorogenic substrates, inhibitor, and inhibitor-like activity-based probes containing biotin or fluorophore. In the next stage of the research, the enzyme preferences for specific amino acid residues in the P1'-P3 'positions were established, which allowed for the synthesis of a substrate-like quenched activity-based probe, which, unlike chemical inhibitor-like activity-based probe, contained a quencher molecule in its structure, but did not have an electrophilic warhead. Due to the high similarity of the specificity profiles of GrB with caspases, the selectivity of the synthesized activity-based probe in complex biological systems was tested. Thanks to the SDS-PAGE technique, the presence of a high concentration of the active form of granzyme B in the YT cell line was detected, as well as its absence in the MDA-MB 231 tumor cell line, which allowed for its use in subsequent stages of research. In the presented doctoral dissertation, an attempt was made to examine the effect of granzyme B on cancer cells and imaging the enzyme in a real time. Studies with the use of the designed substrate-like quenched activity-based probe in combination with flow cytometry and confocal microscopy techniques demonstrated the level of this probe uptake from the culture medium over time, and confirmed the presence of granzyme B inside YT cells. Until now, the biggest problem in studying the activity of granzyme B has been chemical tools with low affinity for the active site of the enzyme, and this doctoral dissertation presents the structures of the molecules, thanks to which it is possible to track the activity of granzyme B *in vitro* and *in vivo*.