DESCRIPTION FOR THE GENERAL PUBLIC

Proteases (proteolytic enzymes, peptidases) are enzymes responsible for protein degradation by performing chemical reaction called peptide bond hydrolysis. They are key players in proper functioning of human organism. Countless studies, performed over the last fifty years, show their fundamental role in almost all biological processes affecting cell differentiation and functioning. Caspases, enzymes that belong to cysteine protease family, are named after their almost absolute requirement for aspartic acid in P1 position of peptide substrates. For almost twenty years these enzymes have attracted the attention of the scientific community because of their involvement in processes called apoptosis and pyroptosis, being two distinct pathways of programmed cell death. These processes are one of the most important cellular events that allow to maintain an appropriate number of cells in human organism, and also to eliminate unnecessary, mutated or damaged cells. All of this has great influence on biological balance. Dysregulation of programmed cell death can lead to many dangerous disorders, such as myocardial infarctions, strokes or cancers. Due to their contribution in organism functioning, apoptosis and pyroptosis have been of great interest for many scientists, trying to discover complicated pathways controlling these processes. Apoptosis and pyroptosis differ from each other, both on molecular level as well as morphologically. The main characteristics of pyroptosis are cell enlargement, membrane disruption and release of proinflammatory cytokines. This process is caspase-1 dependent. On the other hand, during apoptosis, cellular packaging is promoted (the cell decreases its size) and apoptotic bodies, unique for this process, are being phagocytosed. Apoptosis occurs without triggering an inflammation and therefore, it can be said that apoptosis, in contrast to pyroptosis, is an immunologically silent process. Enzyme that is responsible for execution of apoptosis is caspase 3. The newest research revealed that these two types of programmed cell death, despite the fact that they are so different, can be connected with each other. A bidirectional interplay between pathways leading to apoptosis and pyroptosis, and so between caspase 1 and caspase 3, has been recently described by dr Bachovchin. Nevertheless, proposed model is still not full. The main problem, that impedes precise description of events taking place during cell suicide, is the lack of specific tools that would allow to monitor and analyze pathways leading to cell elimination. In case of caspases one of the major obstacles is the fact, that they exhibit overlapping substrate specificity. It means that they recognize similar substrates and so chemical probes, designed basing on peptide sequences of such substrates, allow to monitor activity of few enzymes simultaneously what causes unprecise analysis. The cross-specificity of these enzymes constitutes a major obstacle in performing biological studies. This problem can be solved with the use of specific fluorogenic substrates that contain not only natural, but also unnatural amino acids, what significantly increases the opportunity to distinguish particular enzymes. Unfortunately, in case of such chemical compounds, after peptide bond hydrolysis, the fluorescent group leaves enzyme's active site and therefore fluorescence signal is weak and distracted. This in turn negatively affects the use of this type of molecule for enzyme localization in the cell. Another possibility to investigate enzyme activity in the cell is the usage of irreversible inhibitors which bind tightly to enzyme's active site in 1:1 ratio. This characteristic allows to precisely determine the localization of particular protease. Nevertheless, utilization of strongly electrophilic molecule may cause complete inhibition of its activity, thus preventing further observation of cellular mechanisms. Moreover, in case of inhibitor- based chemical compounds there is no signal amplification as it happens with fluorogenic substrates. Described problems can be solved with proper selection of small molecule chemical probes with certain properties and mechanisms of action. The aim of this project is to design and synthesize selective small molecule chemical markers that will combine the advantages of substrates (signal amplification) and inhibitors (the possibility of enzyme localization). Moreover, biochemical and biological evaluation of obtained compounds is planned, as well as their comparison with commonly used chemical tools. It will allow for direct monitoring of caspase activity in particular cell death mechanisms, such as apoptosis or pyroptosis.