

Atherosclerosis is an inflammatory disease, initiated by subendothelial retention of the apolipoprotein B-containing lipoproteins and their modification to form proinflammatory damage-associated molecular patterns (DAMPs). DAMPs-triggered low-grade inflammation involves main three cell types that participate in development and destabilization of atherosclerotic plaques: endothelial cells, smooth muscle cells and monocytes. Monocyte-derived macrophages undergo maladaptive cellular death programs and by secretion of plethora of proinflammatory factors are considered to be a key drivers of the atherogenesis and crucial players in vulnerable plaque formation.

Therapies based on cholesterol-lowering drugs (statins) have significantly improved the clinical picture, life-span and wellbeing, however, there is still a significant number of patients who does not fully respond to the lipid-lowering strategies. Thus, an additional novel treatment, based on the different mechanisms of action, that ameliorates atherosclerosis progression or improves plaque stability is vitally needed.

One example of such novel, emerging strategies is the targeting the dipeptidyl peptidases (DPPs) in macrophages. In general, the understanding of the significance of proteases action within the cell evolved substantially over the years. They can no longer be recognized as unspecific protein degradation agents, supplying the building material for protein turnover. In contrary, it had become clear that they control essentially all aspects of the cellular life, modifying signaling molecules, their targets, localization and function. Thus, they possess a vital role in both physiology and pathology (e.g. they influence atherosclerotic plaque stability), being an attractive target for drug treatment. Most widely examined is the DPPIV isoform, which inhibitors are now in clinical use to treat type 2 diabetes. Other members of the proteases family include DDP8, DDP9 and fibroblast activation protein (FAP), however they are poorly understood and their biological function remains largely unknown. Importantly, DPP8/9 are abundantly present in macrophage-rich regions of atherosclerotic plaques, accounting for the most of the total DPP activity. Inhibition of DPP8/9 (compound 1G244) reduced activation of M1 macrophages, but not of M2 subtype. Additionally, DPP8/9 inhibition enhanced macrophage apoptosis, pointing to important therapeutic possibilities in reducing of atherosclerotic plaque and/or in the prevention of plaque rupture.

The aim of the presented project is the comprehensive study of the effect of DPP8/9 inhibition on macrophage functions and to assess the anti-atherogenic effect of DPP8/9 inhibition in vivo (apoE-knockout mice model).

We propose the implementation of innovative new tools to describe the role of DPP8/9 in macrophages. We will use the quantitative proteomic approach (iTRAQ) to examine the effect of DPP8/9 inhibition on macrophage proteome and secretome. Methodology progress in the proteomics field has been tremendous over the last decade. Modern proteomic techniques, based on liquid chromatography and mass spectrometry, enable for identification and quantitation of thousands of proteins simultaneously. Such analysis outreaches the classical targeted approaches (Western blot, ELISA), since it is not restricted to the predictable, well known markers of interest (like cytokines). Thus, incorporation of all detectable proteins allows for more broader view of the proteome repertoire, implying its functional consequences. Additionally we will take the advantages of TAILS method to search for the DPP8/9 natural substrates in macrophages. Examinations will be extended by in-depth analysis of the morphology of atherosclerotic plaques, as well as the phenotype and activity of macrophages by means of immunohistochemical, immunoblotting, flow cytometry and RT-PCR methods.