The role of protein prenylation in immune and pancreatic islet β-cells

mgr inż. Daria Kamińska

Supervisor: dr hab. Edyta Gendaszewska-Darmach, prof. uczelni

Second supervisor: dr hab. inż. Katarzyna Błażewska, prof. uczelni

Type 2 diabetes mellitus (T2DM), characterized by chronic hyperglycemia, insulin resistance and immune dysfunction, is one of the world's most significant public health challenges. Improper eating habits-particularly excessive consumption of simple sugars, saturated fats and highly processed foods combined with a lack of regular physical activity play a key role in the development of this disease. Untreated or inadequately controlled type 2 diabetes can lead to serious health complications such as kidney failure, myocardial infarction and stroke, significantly increasing the risk of premature death. Given the complexity of this disease, further research into the molecular mechanisms underlying its pathogenesis is needed. Understanding these pathways is crucial, as they offer promising targets for new therapeutic strategies, both pharmacological and dietary.

In view of emerging reports of a potential link between long-term statin use (inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), a key enzyme that limits the effectiveness of the entire mevalonic acid (MVA) pathway) and an increased incidence of type 2 diabetes, it is crucial to understand not only the role of the enzymes in this pathway, but also the prenylation processes of small GTPases. Inhibition of HMGCR leads to more than just lowering cholesterol levels; it also reduces the synthesis of isoprenoid substrates necessary for post-translational modification of proteins. Most prenylated proteins belong to the Ras family of small GTPases, which play a key role in cellular processes ranging from cell cycle control to vesicular transport. Therefore, understanding the effects of statins on the prenylation of these proteins may be important for elucidating the mechanisms underlying the diabetogenic effects of these drugs.

In this doctoral thesis, an attempt was made to investigate how inhibiting mevalonic acid pathway enzymes, prenyltransferases and Rho-associated coiled-coil forming kinase (ROCK) affects insulin secretion by pancreatic β cells (MIN6 cell line) and mast cell degranulation (RBL-2H3 cell line). In addition, the effect of the inhibitors on the prenylation of selected small GTPases was analyzed: Rab11A, Rap1A/Rap1B, and, in the case of mast cells, also Ras. An assessment of the cytotoxicity of the used compounds against both cell lines preceded all experiments. These studies were carried out at the parent institution, i.e. the Institute of Molecular and Industrial Biotechnology of the Lodz University of Technology. In the second part of the research, the expression level of MVA pathway enzymes and prenyltransferases was measured in peripheral blood mononuclear cells (PBMCs) from healthy individuals and

patients with type 2 diabetes. This research was conducted at The Core Research Laboratory of the Second Affiliated Hospital of Xi'an Jiaotong University in China.

The first part of the study showed that pravastatin and DGBP, used at safe non-cytotoxic concentrations (pravastatin: 10 and 25 μM; DGBP: 25 μM), significantly inhibited insulin secretion under high glucose conditions. At the same time, they caused the accumulation of non-prenylated Rab11A and Rap1A/Rap1B proteins in the cytosol, suggesting that dysregulation of signaling pathways involving these small GTPases may be responsible for the observed reduction in insulin secretion. Risedronic acid (5 μM) was the second compound showing an inhibitory effect against insulin secretion at 20 mM glucose concentration. However, it did not cause a statistically significant reduction in the prenylation of any of the GTPases tested, suggesting that the mechanism leading to reduced secretion may be independent of the prenylation of these proteins. In the case of mast cell degranulation, most of the inhibitors tested contributed to the inhibition of this process. Some of them also caused a decrease in the prenylation of small GTPases, which may suggest that impaired prenylation is responsible for the observed effect in these cases. However, this issue remains poorly understood and requires further in-depth research.

The second part of the study, which was cross-sectional in nature, showed that transcript levels of genes encoding enzymes such as HMG-CoA reductase, farnesyl diphosphate synthase, and farnesyltransferase are significantly increased in PBMCs from people with type 2 diabetes. However, these changes in transcription were not always reflected in translation. Cytometric analysis showed that HMGCR and geranylgeranyl diphosphate synthase protein levels were significantly elevated in the entire population of PBMCs of T2DM patients. Moreover, increased levels of the second enzyme were observed in subpopulations of PBMCs from T2DM subjects: $CD16^+$ monocytes, $CD14^-CD16^+$ non-classical monocytes and in $CD11c^+$ dendritic cells. In the case of farnesyltransferase, a significant difference was noted in the $CD14^+CD16^-$ classical monocytes population – a higher level of this enzyme was found in healthy individuals. The observed changes indicate the potential importance of the mevalonate pathway and its enzymes in the pathophysiology of type 2 diabetes. However, further studies are needed to determine whether the noted changes in enzyme expression have an impact on the phenotype of the affected cells and can provide starting points for in-depth analyses of the molecular mechanisms involved in the disease.

In turn, the analysis of correlations between the enzyme expression level (based on flow cytometry data) and selected clinical parameters revealed some interesting relationships. One was the inverse correlation between the expression level of geranylgeranyltransferase type I in the entire population of PBMCs (both healthy subjects and T2DM patients) and fasting glucose levels. This result may indicate a potential role for this enzyme in regulating glucose metabolism, which requires further research to confirm and clarify the mechanism of this relationship.

In conclusion, the experiments carried out within the framework of the presented dissertation showed that both indirect and direct inhibition of prenylation of small GTPases can affect the processes of insulin secretion by pancreatic β-cells and mast cell degranulation. In addition, a first-of-its-kind cross-sectional study using peripheral blood mononuclear cells from healthy individuals and patients with type 2 diabetes has provided new data on the expression of key enzymes of the mevalonic acid pathway and prenyltransferases in these cells. The obtained results may constitute a valuable basis for further analyses aimed at better understanding the molecular mechanisms responsible for the development of type 2 diabetes and identifying potential therapeutic targets.