The Role of Diaph1 Signaling in cardiovascular autonomic neuropathy in diabetes

Cardiovascular autonomic neuropathy (CAN) impacts up to 91% of patients with type 1 diabetes and up to 75% of patients with type 2 diabetes. With the silent onset, it advances undiagnosed over the years, degenerating heart and blood vessel autonomic nerve fibers. CAN leads to catastrophic consequences such as cardiac arrhythmias, asymptomatic myocardial ischemia, and, without treatment, sudden cardiac arrest (sudden cardiac death). Despite refinements in understanding diabetes and its related complications, the complete picture of CAN's development and progress remains undiscovered. Our project focuses on this novel subject to uncover the causes and potentially reveal new therapeutic solutions in CAN treatment in diabetic patients.

In our study, we plan to decipher the role of the Diaph1 protein (Diaphanous protein homolog 1) in the context of CAN. Diaph1, a member of the formin protein family, is known for its role in actin polymerization and regulation of the cytoskeleton. Emerging evidence obtained via our ongoing research suggests that Diaph1 might be a key player in the development of CAN. RAGE (Receptor for Advanced Glycation End-products) is a transmembrane receptor from the immunoglobulin family, strongly associated with the immune response and inflammation, crucial in developing diabetic complications. It reacts with Diaph1 in the signaling pathway. Our research base is a well-established and commonly employed animal model of pharmacologically induced diabetes, streptozotocin-induced. In this model, the pathological changes observed in tissues most sensitive to hyperglycemia (increased blood sugar levels) mimic the changes in tissues of people with diabetes. Our study cohort includes mice with diabetes three and six months after confirmation of hyperglycemia and controls without diabetes of different genotypes. Not modified – wild type, and genetically engineered using CRISPR-Cas9 technique – Diaph1 knockout, RAGE knockout, and Diaph1-RAGE knockout. The study also includes neurofilament, beta-actin and profilin, which are proteins acting as potential markers of neurodegeneration. Their altered expression may provide information regarding cellular and molecular modifications in CAN.

For this purpose, we plan to utilize state-of-the-art techniques of immunofluorescent staining and immunoblotting – previous experiences with a model allowed for the optimization of the procedures. Employing next-generation sequencing (NGS) will gather vast information on RNA expression differences in heart tissue. We plan to use additional histological staining techniques to visualize the morphological changes occurring in CAN. This in-depth approach may elucidate the role of the chosen proteins in the pathogenesis and progression of CAN. The complex design of the study provides a comprehensive dataset on the topic. It also goes along with the Three Rs principles, as the hearts of the mice come from our other project. That aims to reduce the number of animals and resources required to obtain data and counteract the waste of biological material.

By shedding light on new signaling pathways in CAN, we hope to significantly contribute to comprehending the etiopathogenesis of this condition. The results might pave the way for obtaining more precise diagnostic and prognostic tools and lead to the development of innovative therapies that may extend and improve the quality of life of diabetes patients worldwide.