The role of utrophin in cardiomyopathy in Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a genetic disease caused by mutations in the gene encoding dystrophin - a protein responsible for the connection of the cytoskeleton with the glycoprotein complex located in the cell membrane (mainly in muscle cells). Lack of dystrophin leads to damage of this membrane, and as a consequence degeneration of muscle cells and progressive, irreversible muscle wasting. The disease is inherited in a gender-related manner, therefore it affects mainly boys. The first symptoms appear at the ages between 3 and 5 and are associated with skeletal muscle weakness - difficulties in getting up, climbing the stairs or running. As a result, patients with DMD stop to walk independently and begin using a wheelchair at around 10 years old. In the further course of the disease (around the age of 20), respiratory failure and cardiomyopathy are developed and the cardiomyopathy is the main cause of death of DMD patients. Duchenne muscular dystrophy is an incurable disease. In order to alleviate its symptoms and extend the functional condition of patients, glucocorticoids are used, while no type of pharmacotherapy has any healing properties. In addition, the mechanisms of cardiomyopathy are not fully known. In order to model this disease *in vitro*, human induced pluripotent stem cells (iPSC) are used. Such cells are obtained from easily available somatic cells - usually taken from a skin biopsy or a few milliliters of peripheral blood, and then differentiated into cardiomyocytes, i.e. transformed from stem cells into more specialized cells present in the myocardium. This project aims to investigate the role of utrophin - homologue of dystrophin with a very similar structure and function in the pathogenesis of cardiomyopathy in patients with DMD. Therefore, it is planned to introduce the iPSC line with a mutation in the dystrophin gene and, at the same time, increased expression of utrophin using the CRISPR/Cas9 system, a modern strategy used to modify the genome. As controls, cells with a mutation in the dystrophin without overexpression of the utrophin will be used, as well as controls, initial cells without any genetic modification. The cells will then be differentiated to cardiomyocytes and the electrophysiological properties and activity of ion channels will be compared, as well as the expression of selected small, non-coding RNA molecules - microRNAs, involved in cardiac failure processes. In addition, it is also planned to check the therapeutic potential of cells with overexpression of utrophin after their transplantation into the damaged myocardium of DMD model mice with mutations in the dystrophin- and utrophin-encoding genes. As a standard animal model of DMD *mdx* mice are used, and they have the mutation in the dystrophin gene, while the level of utrophin remains unchanged. In humans, the utrophin is expressed only during the prenatal development, after birth its level disappears, because it is replaced by dystrophin. This difference is also reflected in the severity of symptoms - in *mdx* mice skeletal muscle weakness appears later and moreover, they do not develop so severe form of cardiomyopathy, and their lifespan is not significantly shortened. Therefore, it is believed that utrophin can compensate for the lack of dystrophin and provide a potential therapeutic solution for patients with DMD. Previous studies performed in the context of skeletal muscle regeneration have confirmed the beneficial effect of this protein, while the therapeutic potential of utrophin in the human heart has not been well understood. All the planned experiments and results will allow us to broaden our knowledge of the role of utrophin in the heart, to verify the therapeutic potential of human cardiomyocytes overexpressing utrophin after their transplantation into the heart of model mice, and to search for novel therapeutic approaches in DMD-associated cardiomyopathy.