Duchenne muscular dystrophy (DMD) is a sex-linked genetic disorder caused by mutations in the DMD gene, which encodes dystrophin. Its effect is the complete lack of this protein in the cells, which leads to the gradual deterioration of skeletal muscles, as well as the development of heart failure. Long-term observations of patients with DMD have also shown that the disease is systemic in nature and is associated with metabolic disorders such as insulin resistance and diabetes.

Both in *mdx* mice, used as a model of DMD, and in an in vitro model based on dystrophin-deficient human induced pluripotent stem cells (hiPSCs) differentiated into cardiomyocytes (hiPSC-CM), numerous abnormalities in the electrophysiological activity of cells were observed, including disorders of calcium metabolism. In addition, in the hearts of *mdx* mice, the process of autophagy is enhanced and mitophagy is inhibited, and in skeletal muscles, glucose metabolism is also impaired, both at the level of its uptake and utilization.

One of the potential factors influencing the phenotype of *mdx* mice is microRNA-378a (miR-378a), which is significantly downregulated in the skeletal muscles of these animals, as well as in humans suffering from DMD. Moreover, our team's previous studies showed less muscle fibrosis in miR-378a-deficient *mdx* mice, as well as a functional improvement in the animals' condition. miR-378a is referred to as one of the important regulators of cellular metabolism, being involved in the regulation of both glucose uptake, mitochondrial function, the Warburg effect, as well as the autophagy process. Importantly, previous studies in animal models have shown that miR-378a affects the formation of post-infarction scar and hypertrophic growth of the heart, but its role in human cardiomyocytes, in particularly in the context of the regulation of metabolism and function of dystrophin- deficient cells, has not been described so far.

Taking this into account, the aim of the study was to thoroughly understand miR-378a activity in control and dystrophin-deficient human cardiomyocytes using hiPSC-CM as a research model. These cells were obtained by differentiating hiPSCs using small molecule compounds that regulate the WNT signaling pathway. CRISPR/Cas9 was used to prepare suitable hiPSC lines with the deletion of *MIR378A* (hiPSC miR-378aKO), *DMD* exon 50 (hiPSC DMDex50KO) or both (hiPSC DKO).

The conducted studies show that miR-378a does not affect the electrophysiology of hiPSC-CM, but it plays an important role in the regulation of hypertrophic growth and calcium wave oscillations. Its absence resulted in an increase in the size of cardiomyocytes and a decrease in

the frequency of the influx of calcium ions into the cells. It is also important for glucose metabolism in cardiomyocytes by regulating the level of glucose transporter GLUT1. In particular, the absence of miR-378a resulted in an increase in the level of this transporter, as well as a trend towards increased glucose uptake, but hexokinase and LDHA activity remained decreased. At the same time, a decrease in metabolic activity was observed in hiPSC-CM miR-378aKO in Seahorse XF Mito Stress tests. The lack of miR-378a decreased the level of subunits building the mitochondrial respiratory chain, the mtDNA/DNA ratio, and the level of *TFAM* expression involved in mtDNA replication. A decrease in effective mitophagy and autophagy was also observed in cardiomyocytes lacking miR-378a.

Previous studies indicate that miR-378a may be a potential therapeutic target in DMD. Extending these experiments to the human dystrophic hiPSC-CM (hiPSC-CM DMD) model, reduced miR-378a expression was observed compared to normal cells. Nevertheless, both lack and overexpression of miR-378a had no effect on the tested electrophysiological properties of hiPSC-CM DMD, but significant effects of miR-378a deletion were observed at the level of glucose uptake. In particular, the lack of miR-378a caused a decrease in the amount of glucose uptake and a decrease in the expression of its transporters, as well as an increase in the level of the active form of GSK3β. In both hiPSC-CM DMD and DKO we observed a decrease in *TFAM* and mtDNA/DNA expression. hiPSC-CM DMD showed reduced levels of LC3 I and II in both starvation-induced autophagy and mitochondrial electron chain uncoupling mitophagy assays, while miR-378a deletion in hiPSC-CM DMD reversed these effects. However, the lack of miR-378a had no effect on impaired mitochondrial biosynthesis in DMD cardiomyocytes.

In conclusion, the conducted research allowed us to determine the role of miR-378a in cardiomyocytes and its potential action in Duchenne muscular dystrophy. In the hiPSC-CM model, miR-378a plays a role in the regulation of hypertrophic growth, calcium metabolism, mitochondrial biosynthesis and mitophagy, glucose metabolism and autophagy. Deletion and overexpression of miR-378a did not affect the electrophysiological potential of hiPSC-CM DMD, however, the absence of miR-378a reduced glucose uptake and decreased the level of its transporters in DMD cardiomyocytes. We also observed an increase in the level of LC3 and its lipidated form after mitochondrial electron chain uncoupling assay, suggesting an improvement in mitophagy induction.

The obtained results suggest that inhibition of miR-378a expression in dystrophin-deficient cardiomyocytes restores the balance between mitochondrial biosynthesis and mitophagy. Combined with previous observations showing improved exercise capacity and metabolism in

mdx mice with the addition of miR-378a down-regulation, the present results point to miR-378a as a potential target for disease-modulating therapy in Duchenne muscular dystrophy.