Molecular mechanisms of heart failure in Duchenne and Becker muscular dystrophy

Duchenne muscular dystrophy (DMD) is an incurable disease that affects about 1 out of 5,000 boys. It starts to develop in boys aged 2-5 years, causing loss of ambulation at early teenage and inevitably leading to death, nowadays mostly in the second or third decade of life. DMD is caused by mutations in the dystrophin gene (*DMD*) located on the X chromosome. It is the largest of human genes, and the number of known mutations leading to the total lack of this protein is now over 7,000. Because women have two X chromosomes, and men only one, the disease manifests itself in boys, in a situation where the mother transfers to her son a mutated X chromosome. However, in a large number of cases (about 30%) the disease is caused by *de novo* mutations.

The lack of dystrophin, necessary for the proper functioning of skeletal, respiratory (diaphragm) and heart muscles, and to some extent the nervous cells, leads mainly to limb muscles damage, and therefore mobility problems become apparent first. The progression of the disease, which can be partially prevented by the use of corticosteroids, leads inevitably to the loss of walking ability at the age of 10-15 years. The involvement of respiratory muscles also impairs breathing, which is now supported by mechanical ventilation, but the lack of dystrophin causes damage to myocardial cells, the development of cardiomyopathy and, as a consequence, heart failure.

Besides corticosteroids, there are no medications effectively inhibiting the progression of the disease. The cardiac drugs applied also do not improve efficiently the myocardial function of patients with DMD. For these reasons, there is a need to thoroughly investigate the mechanisms responsible for the development of cardiomyopathy in DMD. In the submitted project, we will undertake this task using the strategy of induced pluripotent stem cells (iPSC) - a method developed by prof. Shinya Yamanaka from the University of Kyoto in Japan, awarded the Nobel Prize for this discovery in 2012. This method involves obtaining stem cells capable of differentiating (transforming) into virtually all cell types of the body. Using easily available cells (eg white blood cells), through appropriate (transient) genetic manipulations, we can transform them into iPSC cells, and these then differentiate into cardiomyocytes - contractile myocardial cells, as well as endothelial cells (forming vessels in the heart) and fibroblasts (another important cell population in the heart). Obtaining thus the iPSC and then the heart cells of patients with DMD, we will examine their properties (global gene expression) and compare them with the healthy cells. We will investigate also the properties of similarly obtained cells from patients with Becker dystrophy – the rarer and milder form of the disease, in which the mutation in dystrophin gene does not lead to total loss of the protein but results in production of shorter version. We will investigate also the cells of mothers – carriers of the *DMD* gene mutation. In our studies we will use also the latest technique of genetic engineering - the so-called gene editing that allows for precise repair of mutations. The obtained data will be compared with the results of investigations carried out on the DMD animal model - the so-called *mdx* mice, which also lack dystrophin, and moreover, using our recently developed models, we will check how the lack of additional genes affects the development of cardiomyopathy in *mdx* mice.

Obtained results from iPSC and animal models will be the basis for a cell therapy trial of cardiomyopathy relying on the administration of human functional cardiomyocytes (containing dystrophin) to *mdx* (immunodeficient*)* mice, as well as cardiomyocytes additionally enriched in genes whose protective properties have been discovered in previous studies and those which will prove to be a useful therapeutic target based on analysis of cells differentiated from iPSC.

To our knowledge, this will be the first such comprehensive study of the mechanisms of cardiomyopathy in DMD. We expect that the results will allow a better understanding of the processes leading to the heart failure deaths of DMD patient, and thus allow in the future to find new ways of at least partial treatment of this dramatic disease.