Targeting microRNA-378 in a mice model of Duchenne muscular dystrophy – a weapon against hallmark symptoms of the disease?

Duchenne muscular dystrophy (DMD) is a genetic disorder that affects approximately 1 in 5000 boys and is manifested by the progressive and at the same time irreversible muscle wasting. It was firstly described in 1861 by the French neurologist Guillaume Benjamin Amand Duchenne, but its direct cause was identified only over 100 years later. Nonetheless, DMD still remains an incurable disease. The first noticeable symptoms of the disease appear already at the age of 2-3 years and are related to the problems with walking and running, whereas at the age of 12-15, the patients are no longer able to walk alone.The disease progresses very rapidly, eventually leading to cardio-respiratory failure, which is the leading cause of death of patients in their second or third decade of life.

DMD represents one of the most severe forms of muscular dystrophy and its etiology is associated with the presence of numerous mutations in the dystrophin coding gene. Dystrophin is the structural protein of all muscle cells thereby it stabilizes the muscles during contraction. The lack of functional dystrophin makes the muscles extremely sensitive and prone to mechanical damage what further leads to their necrosis and inflammatory reaction. At the same time, muscle stem cells, so called satellite cells, get activated in order to regenerate the damaged muscles. Unfortunately, continuous cycles of damage and regeneration with sustained inflammationover the years leads to the exhaustion of satellite cells pool. This in turnresults in muscle atrophy and replacement of muscles with fatty and fibrous tissues.

Glucocorticoids, which act mainly as an anti-inflammatory agents, are the only available treatment for patients with DMD currently. Nevertheless, despite the beneficial effects in e.g. prolonging ambulation, their daily administration is associated with many side effects. Therefore, it is necessary to search for the new factors influencing the progression of this disease. Recent studies suggest that in addition to the direct, muscle-related symptoms of the disease, also disturbances in systemic glucose and lipid metabolism can contribute to the deterioration of patient's condition. Therefore, it can be envisaged that factors influencing muscle-related symptoms of the disease and modulating systemic metabolism can significantly affect the progression of DMD. One of the candidates is microRNA-378 (miR-378), a small non-coding RNA molecule that is able to regulate the expression of various genes. Literature data indicate a significant role of miR-378 in processes related to the glucose, lipid metabolism and muscle cells biology. Interestingly, the increased level of miR-378 in DMD patients has been demonstrated, and our preliminary studies suggest that the deficiency of miR-378 in the mouse DMD model, so called *mdx* mice leads to the improvement of the dystrophic phenotype by e.g. increased running capacity. Nonetheless, it is still unrecognized if the changes are solely driven by improved muscle functionality itself, systemic changes in the metabolism, or both.

Therefore, it the frame of this project we would like to extend our previous research on the role of miR-378 in the progression of DMD in a mouse model of this disease. Our goal is to verify the hypotheses, in which we assume that the improvement of the dystrophic phenotype in mice additionally lacking miR-378 is due to the both changes in muscles functionality and systemic modulation of metabolism. In addition, we propose that the administration of fisetin, a plant polyphenol, will have a beneficial effect on the dystrophic phenotype. Fisetin inhibits miR-378, but also has a number of anti-inflammatory and cytoprotective properties, suggesting its potential application as a factor ameliorating the progression of the disease primary in *mdx* mice, but potentially also in DMD patients.

To address the hypotheses we will conduct experiments on the mouse model of DMD, *mdx* mice and also on *mdx* mice additionally lacking miR-378. We will conduct the analysis of muscle functionality by performing grip strength assay as well as muscle contraction and mechanical stability measurements. We will also perform a series of experiments related to the investigation of changes in glucose and lipid metabolism, analyzing them both in the muscles and in the liver. In order to check the effect of fisetin on the dystrophic phenotype, we will deeply investigate its impact on muscle functionality and metabolism as described above and we will in detail examine the changes associated with muscle degeneration and regeneration. In addition, we will perform a global analysis of gene expression from muscle and liver tissues to search for the potential mechanisms of fisetin action.

The results of this project will facilitate the in-depth understanding of the role of miR-378 in muscle functionality and modulation of glucose and lipid metabolism in the mouse model of DMD. We do believe to uncover the answer for many bothering questions. Furthermore, proposed administration of fisetincould potentially provide a novel nutritional, if not therapeutic, strategy to attenuate hallmark symptoms of the disease.