Role of unconventional myosin VI in development of cardiomyopathy: New insights into understanding of function and dysfunction of cardiac muscle

The proposal addresses the processes vital to human health, namely cardiac muscle function and the development of cardiomyopathy (CM), one of the main causes of human death worldwide. CM affects cardiac muscle and is associated with heart enlargement and weakening, cause by the impairment of its pump function. Hypertrophic cardiomyopathy (HCM) is the most common primary CM in the population worldwide, characterized by the abnormal cardiac growth, leading to thickened heart walls, with particularly left ventricle affected. It is also responsible for sudden cardiac death, often occurring in the athletes. The other type is dilated CM (DCM), which is characterized by the ventricle chamber dilation with normal or decreased wall thickness. In most cases, cardiomyopathies are caused by a single mutation within genes encoding muscle proteins, with the most prevalent of β-cardiac myosin heavy chain, MYH7. Despite intensive research on understanding the mechanisms behind CM development, the etiopathology of this severe, life-threatening illness has not been yet fully understood. In the course of worldwide studies new players that could be involved with heart dysfunction are being identified, and such a novel player could be a unique actin-based motor, unconventional myosin VI (MVI).

MVI belongs to a large class of unconventional myosins, which contrary to muscle myosin isoforms do not form filaments and act either as cargo transporters or factors regulating organization of numerous subcellular compartments, thus being involved in a variety of cellular processes. Though they are present in cardiac muscle, very little is known about their functions in the heart. MVI, unlike all other known myosins, moves towards the minus end of actin filaments, implying its distinct role in the cells. First indication about MVI involvement in heart function came from the study showing that a mutation within a gene encoding human MVI was associated with mild symptoms of hypertrophic cardiomyopathy. Followed this, we performed pioneer studies, which demonstrated that in murine hearts MVI localized to the sarcoplasmic reticulum and intercalated discs, and its presence was important for organization of these myocyte compartments. We also demonstrated that the observed heart enlargement of mice lacking MVI (Snell's waltzer, MVI-KO) was already observed in E14.5 embryos and continued throughout the animal life. Moreover, in newborn mice the level of proteins involved in proliferation was elevated, indicating that lack of MVI may activate different signaling pathways, possibly associated with hypertrophy and leading to heart dysfunction.

Based on the available data, including our own preliminary results, **we hypothesize that MVI plays an important role(s) in the heart and its lack leads to the development of cardiomyopathy, progressing with age.** To understand the mechanisms behind the observed MVI-associated heart enlargement, we are going to perform studies on MVI-KO mice and heart explants from patients diagnosed with hypertrophic (HCM) and dilated (DCM) cardiomyopathies; the latter will be performed in collaboration with State Research Institute Centre for Innovative Medicine in Vilnius, Lithuania. In order to analyze whether the differences observed in murine hearts progress with age, the experiments will be performed on hearts retrieved from mouse at different age, from embryos E14.5 till adult 12-month old male mice. The controls will be heterozygous littermates (WT). The studies will be performed at the molecular, cellular, tissue and whole organism level, with the use a broad range of modern experimental techniques, including functional echocardiographic studies.

The following experimental tasks will be addressed to verify our working hypothesis:
Task I. Evaluation of the level and localization of MVI in heart explants from DCM and HCM human hearts. The planned experiments should provide additional evidence about association of MVI with CM in humans.
Task II. Analysis of the regenerative potential of MVI-KO myocardium-derived mesenchymal/stromal cells (H-MSCs). Since recent studies indicate that a fraction of H-MSCs has ability to proliferate and differentiate, we plan to examine whether MVI could be involved in regulation of regenerative potential of these invaluable cells. This is of special interest as there are several studies pointing at the potential suitability of H-MSCs in repair of diseased hearts thus opening new therapeutic possibilities.
Task III. Morphometric and functional analysis of hearts of MVI-KO mice. We will analyze myocardium and cardiomyocyte dimensions (including their compartments) as well as the myocyte number and alignment within the examined hearts. The more, we will perform echocardiography on the MVI-KO mice at different age. These analyses should enable to conclude whether the described MVI-KO heart enlargement and possible dysfunction progress with age as well as to reveal whether they are of a dilated or hypertrophic phenotype.

The obtained data will provide novel information on a role of MVI in the heart function and development of cardiomyopathy. Also, we believe that understanding the role of MVI in the heart will allow us to get more insight into the novel mechanisms of CM. It is plausible that MVI could be considered as a cytoprotective protein important for heart function, and in the future could be considered as a potential therapeutic target.