All cells of Eukaryotic organisms contain a complex network of polymeric proteins which form cytoskeleton. An important part of the cytoskeleton are actin filaments, which form various structures responsible for many vital functions of the cell. Actin maintains cell shape and tension, participates in cell migration, adhesion, cell division, intracellular transport. Such functional diversity requires that actin can be rapidly organized into a large variety of structures. Actin filaments assemble from globular subunits into two-chain elongated polymers, which are very dynamically elongating and shrinking at both ends with different velocities. These filaments are arranged into various structures by interactions with a huge number of actin-binding proteins. In addition, interactions of actin filaments with myosin motor proteins drive contraction and cell trafficking. Among the actin-binding proteins, there is one special family of proteins called tropomyosins, which polymerize along the filament to stabilize the filament and to control actin interactions with other binding proteins.

During oncogenic transformation and spreading of tumors to distant places (metastasis), actin cytoskeleton undergoes large changes. One of the reasons is that the transformation processes change the level of actin-binding proteins. The levels of several types (isoforms) of tropomyosin were shown to be significantly reduced in different tumors. One of the mechanisms which may lead to changes in actin cytoskeleton in cancer cells due to decreased levels of some tropomyosin isoforms is deregulation of actin-binding proteins which determine actin filament dynamics.

Recent studies by Dr Beneš's research group from Masaryk University in Brno found for the first time that the level of tropomyosin isoform Tpm2.3 was significantly reduced in metastatic osteosarcoma (OSA) cells. Tpm2.3 was not studied so far, therefore we do not know how it affects actin cytoskeleton in general and in OSA cells in particular. OSA is a malignant, highly aggressive bone tumor, affecting mostly children and young adults. To fight this disease, it is important to find molecular markers, which could predict the prognosis of the patients and be targeted by therapeutic drugs. The main question is whether Tpm2.3 can be a prognostic marker or therapeutic target in OSA.

The aims of this research project are: (1) uncovering the molecular mechanisms of actin filament regulation by tropomyosin isoform Tpm2.3, (2) finding the role of Tpm2.3 in maintaining actin filament structures and mechanic properties of non-metastatic and metastatic OSA cells, (3) finding out whether Tpm2.3 can affect sensitivity of cancer cells on therapeutic drugs.

The project will be executed at Kazimierz Wielki University in Bydgoszcz, Poland and at Masaryk University in Brno, Czech Republic. The Polish group will analyze functions of Tpm2.3 on the molecular level. They will produce Tpm2.3 and other tropomyosin isoforms using molecular biology methods. The regulation of actin filament interactions with several actin-binding proteins, which are responsible for dynamics of the cytoskeleton, cell migration and contractility, will be studied using various biochemical methods. This group will also construct specific antibodies, which will allow to localize Tpm2.3 in non-metastatic and metastatic cells. The Czech group will prepare OSA cell lines with altered levels of Tpm2.3 and will analyze phenotype of these cells and their metastatic abilities. Using cell cultures and advanced microscopy methods, the Czech group will study localization of Tpm2.3 in malignant cells, effects of Tpm2.3 on actin cytoskeleton organization and mechanical properties of the cells. They will also analyze response of OSA cells to therapeutic drugs.

Implementation of the project will give us new information on the role of Tpm2.3 in physiological and pathological processes and will give important clues whether Tpm2.3 can be used as a specific marker in OSA.