This project concerns biochemical characterization of newly discovered actin isoform, actbl2 and its involvement in tumor cell motility.

Both normal and tumor cells have the **ability to move within tissues**. In the case of normal cells only part of them has migratory potential, which is strictly controlled by surrounding environment. Protruding of normal cells is under some conditions needed. For instance if skin is wounded activated fibroblasts residing in skin move towards the wound and together with other cells close it. In the case of metastasizing malignancies, tumor cells free themselves from controlling signals coming from surrounding environment and other cells and gain ability to move/invade. After reaching by tumor cells blood vessels, they circuit within blood until they adhere to the wall of blood vessels and go through it into neighboring tissue. Next the cells start to divide creating a metastasis. So, at least part of tumor cells possess migratory potential. That's why it is so important to understand completely processes responsible for motility of cancer cells and thus generating metastases.

Proteins building cell skeleton, in other words cytoskeleton, can be divided into three groups: microfilaments, intermediate filaments and microtubules. While microtubules are responsible for transporting cells organelles and proper cell division and intermediate filaments are crucial for cells shape, **microfilaments are responsible mainly for cells motility. These last structures are consisting of actin**. Actin monomer has an unique ability to create long polymers, which can align in parallel and span the whole cell. More than 200 different proteins directly control generation of actin polymers and their fragmentation. These dynamical changes are responsible for cells movement. While at one end of the cell new microfilaments are formed in the other part of the cells actin filaments are falling apart. What is interesting, actin plays role not only in cells motility but also in several crucial for survival of the cells processes such as e.g. gene transcription in the cell nucleus. Up to very recently only 6 types, in other words isoforms, of actin were known. α skeletal actin builds basic functional element of muscle cells – sarcomer. Myosin, another actin cytoskeleton protein, filaments by sliding over actin filaments generate power stroke for muscle contraction. α cardiac actin is responsible for heart contraction, whereas α smooth actin and γ enteric actin take part in contractions of smooth muscles. Expression of these four actin isoform is thus restricted to certain tissue type – muscles. Other two actin isoforms are β and γ cytoplasmic actin. These isoforms are present in every cell type, although in muscle cells in smaller amounts in comparison to muscle actin. Although the difference in amino acid sequence between β and γ cytoplasmic actin is smaller than 5%, they can form different structures and bind different proteins, what results in varying functions in the cells.

In the last few years there were published data implying presence in some tumor cells of seventh actin isoform, actbl2. These studies applied mass spectrometry, a very sensitive method allowing for detection of fragments of proteins and later their identification. Deciphering of human genome allowed for identification of a gene coding for actbl2. Realization of previous grant „The role of nuclear localized gelsolin in cell migration", founded by the Foundation for Polish Science within HOMING Plus Programme gave several interesting results. We found out that in part of tested human melanoma cell lines there is present this newly discovered, seventh actin isoform. Studied cell lines exhibited varying amounts of actbl2 and different migratory potential. Bioinformatical analysis revealed actbl2 is very similar to cytoplasmic β actin. Thanks to molecular biology techniques we have already shown actbl2 similarly to other actin isoforms is able to form polymers, in other words microfilaments. Thus, it means it can be important for melanoma cells motility.

It is important to mention here, that **melanoma, a pigment cells tumor** is one of the most difficult to cure malignancies. Although only ca. 4% of skin tumors are melanoma cases, more than 80% of deaths caused by skin tumors are due to melanoma. Therefore, it is highly important to understand the biology of melanoma tumor cells in order to find an effective treatment against this quickly progressing malignancy.

According to information presented above and the fact, that there are no studies concerning biochemical and functional characterization of actb2 this project focuses on these aspects.

The work plan of this project includes:

- Biochemical characterization of actbl2.

1. We will generate recombinant actbl2 in a special expression system suitable for actin production. Usually researchers produce human proteins in bacteria. But in the case of actin it is not possible, because actin needs special proteins to fold into an appropriate three-dimensional structure (conformation). That's why we will use a system based on insect cells, which are very good for production of human actin.
2. Having recombinant actbl2 we will conduct several biochemical tests in order to e.g. estimate whether actbl2 polymerizes better or worse than other actin cytoplasmic isoforms or whether actbl2 binds the same proteins as other actin cytoplasmic isoforms and what is the kinetics of these interactions.
3. Obtained recombinant protein actbl2 will allow us to make an attempt to grow crystals of it. Analysis of actbl2 crystals would help to get information about tertiary structure (conformation) of this newly discovered actin isoform.

- Understanding the role of actbl2 in melanoma cells migration and invasion.

1. We possess human melanoma cell lines with varying amounts of actbl2. In order to check the influence of actbl2 on cells motility we want to decrease the amount of actbl2 in the cells with naturally high amounts of actbl2 with the help of molecular biology techniques. On the other way round, having cell lines with naturally small amount of actbl2 we plan to increase the amount of this seventh actin isoform also by using molecular biology techniques. Upon these manipulations we will test these cell lines for their migration ability under 2D and 3D conditions. In this way we will estimate whether decreasing/increasing amount of actbl2 leads to grow or decline in motility of melanoma cells.
2. By applying special cell dying techniques we will check how newly discovered actin isoform localizes within cell and with which proteins, binding "classical" actins, it occupies the same location. Results of this part of the project will give us information about signaling pathways controlling dynamics of e.g. formation of polymers by actbl2 and thus cell motility.

3. We plan also to tag proteins in a special way and then to separate mixture of dyed proteins obtained from melanoma cells in an electric field. This analysis will help us to characterize modification of actbl2 after being synthesized, these modification are commonly called post-translational modifications. It happens in response to signals coming from surrounding environment and from cell itself. Analysis of these modifications will give us valuable knowledge of e.g. how the amount of actbl2 is regulated in the cells.

Summarizing, the results of this project will give knowledge of newly discovered, not studied yet in detail seventh actin isoform, actbl2. This protein could influence cell motility of melanoma cells, highly metastatic malignancy.

For the purposes of this project we will use modern techniques of molecular biology, special systems for productions of recombinant proteins, several biochemical assays and advanced cell cultures of melanoma cells. This project will be conducted at the Cell Pathology Department of the Faculty of Biotechnology, University of Wroclaw, which is a part of the Wroclaw Centre for Biotechnology consortium. This consortium holds the prestigious status KNOW (National Centre for Scientific Lead) for the period 2014-2018. This project creates a new job position of post-doc type. One of the tasks will be realized in the collaboration with the German Partner.