Functional diversification of actin isoforms β and γ in cancer cells migration process.

Cell migration plays an important role in many physiological and pathological processes such as embryogenesis, wound healing, angiogenesis and metastasis. A better understanding of the molecular mechanisms of cell locomotion during these events is therefore of high clinical relevance. Mesenchymal and ameboid type of movement are two main modes of cancer cells migration. Mesenchymal cell motility is Rac- and protease-dependent and associated with occurrence of filamentous actin rich protrusions such as lamellipodia and invadopodia. Ameboid mode of motility is Rho/ROCK kinases-dependent. Cells moving in this way are round and form numerous membrane blebs. Reorganization of the actin cytoskeleton is the primary mechanism of cell motility regardless of cell migration type. Actins are eukaryotic proteins, which are involved in diverse cellular functions including muscle contraction, cell motility, adhesion and maintenance of cell shape. Cytoplasmic, non-muscle actin isoforms β and γ are ubiquitously expressed and essential for cell functioning. They differ only by four amino acids located at positions 1-3 and 10, near the N-terminal region of actin molecule. Proportion of these proteins varies and depends on the cell type. Functions that they fulfill and their subcellular localization are still in the phase of research. The aim of this project was to establish if there exist a functional diversification of cytoplasmic actin isoforms β and γ in cancer cells migration process.

In this project human cancer cell lines differing in mode of movement were used – breast cancer MDA-MB-231 cells, which move in mesenchymal way and colon cancer LS174T cells, that migrate in ameboidal mode. Our data demonstrated a similar level of β and γ actin isoforms in both cell lines. In LS174T cells microfilaments were observed as a cortical ring under the membrane, whereas actin cytoskeleton of MDA-MB-231 cells was more dispersed, without prominent stress fibres. Both actin isoforms were mainly concentrated in the submembranous region of mesenchymally as well as ameboidally migrating cells. After stimulation with EGF increased protrusions formation activity was observed in both lines. MDA-MB-231 cells formed flat lammellipodia and many invadopodia and LS174T cells created small blebbs.

The next aim of this study was to determine the effect of β and γ actin overexpression on the migration capacity and actin cytoskeleton organization of both examined cell lines. Cells were transfected with plasmids: pAcGFP-C1-β actin or pAcGFP-C1-γ actin, containing cDNA for β or γ actin isoform. In both kinds of cells, overexpression of β as well as γ actin caused increased migration and invasion abilities. Actin overexpressing cells presented also the

elevated filamentous to monomeric actin ratio. Overexpressed actins in both : MDA-MB-231 and LS174T cells were observed mainly in filamentous form and localized in the submembranous region of the cell. In mesenchymally migrating cells especially near to the leading edge and on the tips of pseudopodia. In ameboidally migrating cells increased blebbs formation activity was observed.

Furthermore involvement of cytoplasmic actin isoforms in invadopodia formation was examined. Invadopodia are actin-rich protrusions formed by mesenchymally migrating cancer cells. They are mainly composed of actin, actin-associated proteins, integrins and proteins of signaling machineries. These protrusions display focalized proteolytic activity towards the extracellular matrix. It is well known that polymerized actin is present in these structures, but the nature of the actin isoform was not studied. Obtained results show that both cytoplasmic actin isoforms, β and γ, are present in the invadopodia of MDA-MB-231 breast cancer cells cultured on a 2D-surface. It was also demonstrated using isoform specific antibodies and expression of the fluorescently-tagged actin isoforms that invadopodial structures formed by the cells in 3D-collagen matrix also contain β and γ actin. Additionally, after simultaneous expression of differentially tagged β and γ actin in cells, it was shown that the actin isoforms are present together in a single invadopodium. Cells with an increased level of β or γ actin, display a similar increase in the number of invadopodia and in the surface they occupy compared to control cells.

In conclusion, obtained results clearly suggests that cytoplasmic actin isoforms β and γ are involved in cell migration and invasion of both mesenchymally and ameboidally migrating cells. These data suggest also that level of expression of both actin isoforms has an impact on cancer cell motility. Additionally, both cytoplasmic actin isoforms are part of the filamentous actin present in invadopodia structures formed by mesenchymally migrating cancer cells.