

TO SEE A SIGNAL – MICROSCOPIC TECHNIQUES AS A TOOL IN SIGNAL TRANSDUCTION STUDIES.

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Different microscopic techniques were applied to evaluate experimentally induced changes in actin cytoskeleton of glioma C6 cells, and to compare changing status of this cytoskeleton to the migration and adhesion mode, morphology and calcium response. We compared 4 groups of examined cells: 1- control cells, 2- cells with stimulated nucleotide receptor (P2Y₂), 3- that one with inhibited RhoA dependent kinase (ROCK) by – Y-27632, 4- cells treated with ROCK inhibitor and then with P2Y₂ receptor agonist - UTP. Such an experimental conditions allowed us to investigate the crosstalk of two basic signaling pathways regulating the functional state of actin cytoskeleton, namely RhoA and calcium pathways. To evaluate molecular mechanism of this crosstalk, activity of two actin-binding proteins – cofilin and myosin II was studied. Their phosphorylation level was measured by immunofluorescence analysis. The colocalization of these proteins with actin filaments was analysed using confocal microscopy and Fluorescence Lifetime Imaging Microscopy to detect FRET between examined structures.

The effects observed on the molecular level are visible also in the cell morphology, migration, adhesion, cell surface mechanical properties and calcium response. To control cell shape, we studied changes of 3D cell shape by confocal microscopy. Morphological changes were also observed in vivo, and by scanning electron microscopy (SEM). Cell migration was examined by direct cell tracking (about 8 hours in each single experiment) using DIC Nomarski contrast microscopy. The IRM (Interference Reflection Microscopy) technique was applied to study area of cell adhesion. Atomic force microscopy was used to compare cell surface stiffness. Calcium response after stimulation of nucleotide receptors was imaging on subcellular level by Fura-2 ratiometry on custom build fluorescence microscope [1].

We have observed that in glioma C6 cells ROCK inhibition decreases cofilin and myosin II phosphorylation, induces changes in the distribution of examined proteins and their colocalization with actin filaments. These changes cause: statistically significant rounding of the cell, measured in volume to area ratio as well as cell height, changes in cell surface structure, increased speed of cell front movement as well as increased cell tail adhesion. Increasing in local cell surface stiffness was noted. We have found that effect of ROCK inhibitor can be compensated by calcium signal [2].

We suggest that two examined signaling pathways may act synergistically or compensate the inhibition of one of them by the activation of other.

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