THE MULTIDIMENSIONAL ANALYSIS OF CELL BEHAVIOUR

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Introduction. The cell motility assays became an important step for trial of new developed anti-tumor drugs and compounds [1, 2]. Set of experiments was performed with microtubule affecting drugs on a model population of 3T3 cells. This study is focused on the complex analysis of cell population behavior that can be further used to develop method for evaluation of drugs in preclinical trials.

Methodology. The behavior of 3T3 cells was analyzed in two independent assays: cell migration from the monolayer into wound scratched on the coverslip ("wound healing" assay) and random cell motility ("stochastic migration") on substrate. Three drugs with different mechanism of interaction with microtubules were used at nanomolar concentrations for microtubule stabilization: nocodazole, taxol and vinorelbine. Wound healing and random motility assays were performed according to classical protocols. Two parameters of wound healing were measured: wound area and closure rate. For random motility assay length of tracks for individual cells and pattern distribution were analyzed. All measurements were done manually using FIJI/ImageJ and ZEN 2012 software tools.

Results and discussion. Wound healing assay show that cells extended into the empty area linearly with constant speed. This speed decreased under the action of all drugs at a concentration of 300 nM, while no significant effect was observed at a concentration of 100 nM of Nocodazole and Taxol. In random cell motility assay cells demonstrated large variability of motion. The most numerous group travelled during 8 hours for 20-50 um. All drugs applied at 100 nM did not alter random cell motility (length of individual tracks did not change), however induced more frequent turnover of migrating cells. At 300 nM for all drugs tested rate of migration decreased and often cells for many hours undergo reversible motions in the limited space.

Cell spreading was third assay. In control majority of cells undergo rapid radial spreading (within 5-10 min). Under the action of 100 nM of all drugs alterations of spreading were insignificant, however the rate of radial spreading slightly decreased. Under the action of 300 nM the duration of period between primary attachment and start of radial spreading increased significantly. Also rate of radial spreading decreased and cell polarization (formation of stable edges) occured more slowly. The preliminary evidence show that Vinorelbine may be more effective however it seems to be more toxic effect in comparison with other drugs.

Conclusions. Effect of low doses of MT inhibitors on cell motility could be determined by different approaches using high-throughput microscopy. Relative sensitivity of approaches could be ranked as follows: random motility test is the most sensitive one. Analysis of cell spreading is similarly sensitive, however data analysis required is more sophisticated. Wound healing assay demonstrate minimal sensitivity especially in the case of low doses when rate of healing could be even higher compared to control cells. Further studies will be conducted to determine cell sensitivity to each drug more precisely.

References.

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