CHEMOTHERAPEUTICS' EFFECTS ON CELL SPREADING AND MOTILITY VIA REGULATION OF MICROTUBULE BINDING PROTEINS

S.Kauanova[1], A.Kakpenova[1], C.Gilman[2*], I.Vorobjev[3]

[1]NURIS, Nazarbayev University, Astana, Kazakhstan; [2]School of Science and Technology, Nazarbayev University, Astana, Kazakhstan; *chgilman@nu.edu.kz; [3]Lomonosov Moscow State University, Moscow, Russia

INTRODUCTION.

Cell motility is essential to life – it is an important feature of development, wound healing, immune response, metastatic growth and many other processes. 40 years ago it was shown that polarized morphology and ability to crawl on a substrate of animal cells largely depends on microtubules (MTs) (Vasiliev et al., 1970; Gail, Boone, 1971). Microtubule-targeting agents (MTAs) are extensively used for the treatment of various cancers, and their effectiveness is mostly attributed to the ability to inhibit mitosis and disrupt tumor vasculature by regulating MT dynamics. Several steps of the mechanism of MTA effect on MT structures have been proposed:

- MTAs, both MT-stabilizing and MT-destabilizing drugs, tend to act or regulate the plus end of MTs
- MT plus ends serve as sites of accumulation of a diverse group of factors known as MT plus end-tracking proteins (+TIPs).
- core components of the +TIP network are the members of the end-binding (EB) family, which autonomously recognize the stabilizing cap at growing MT ends.
- EB proteins strongly bind to polymerizing MT plus ends and affect MT dynamics

AIM AND OBJECTIVES.

Our project will test the hypothesis that MTAs will specifically regulate microtubule interactions with EB proteins, thereby regulating MT dynamics.

Our strategy is to quantify MTA effects on MT structures, cell spreading and cell motility while controlling EB1 and EB3 expression during high-resolution time lapsed imaging.

RESULTS.

The preliminary results are summarized in Fig. 1. Long straight MTs appear in some cells already at the phase of blebbing (B), and at the stage of radial spreading nascent lamella is always filled with long MTs (E).

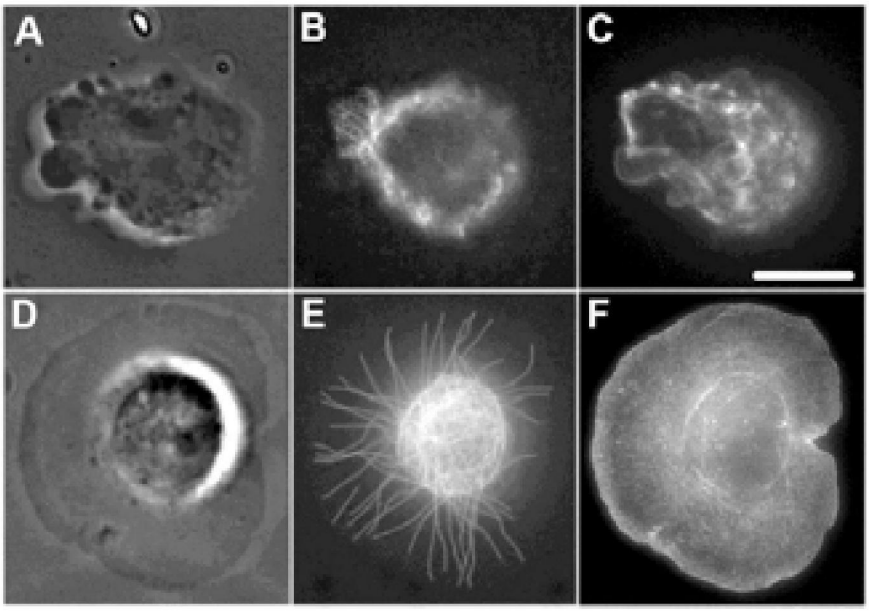

Figure 1. *Fibroblast cytoskeleton organization on early stades of cell spreading.*

A-C - Cytoskeleton organization in a blebbing cell 10 min after plating on the coverslip. A - phase contrast, B - tubulin, C - actin. D - F - Cytoskeleton organization in a cell with radial lamella 15 min after plating on the coverslip. D - phase contrast, E – immunofluorescence, microtubules, F – actin fluorescence with phalloidin. Scale bar 10 μm.

CONCLUSIONS.

We have demonstrated that we are able to quantify morphological and MT dynamics in real-time using high-resolution microscopy. Next we will express EB1 and EB3 cDNA or shRNAs in the presence of MTAs to test our primary hypothesis.

ACKNOWLEDGEMENTS.

Facilitation of the collaboration was due to the kind efforts of Dr Ken Alibek, to whom we are grateful.