Laboratory of Biology of Cytoskeleton

Modulation of microtubule organization, microtubule proteins, signal transduction

Pavel Dráber

pavel.draber@img.cas.cz

The long-term research programme of the laboratory has been focused on studying the structure-function relationships of microtubule (MT) proteins and their interactions with other cytoskeletal elements in cells under normal and pathological conditions. The organization of dynamic MT networks is controlled by microtubule organizing centres (MTOCs). One of the key components of MTOCs is γ-tubulin, which is necessary for nucleation of MT. Current work focuses on the understanding of the modulation of MT properties by signal transduction molecules, the function of γ-tubulin forms, and molecular and functional characterization of regulators of microtubule nucleation. To address these questions, techniques of molecular biology, biochemistry and immunology are being used, as well as a variety of microscopic techniques, including live cell imaging using TIRF microscopy. Our results demonstrate that rearrangement of microtubules in activated mast cells depends on activity of the stromal interacting protein 1 (STIM1) and that Ca^{2+} plays an important role in the formation of microtubule protrusions in activated cells. We have also shown that non-receptor protein tyrosine kinases play an important role in MT nucleation. We have demonstrated that prediction of epitope exposure on microtubules by means of homology modelling combined with site-directed antibodies can contribute to better understanding of the interactions of microtubules with associated proteins. Finally, we have shown that ectopic expression of tubulins may represent a novel marker in pathobiology of glioblastoma multiforme, the most common and deadliest form of primary brain cancers.

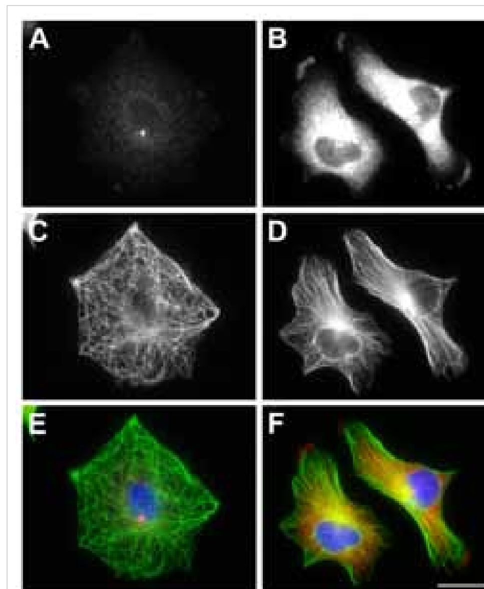

Fig. 1. Different subcellular distribution of γ-tubulin in fibroblasts and glioblastoma cells. Mouse fibroblasts 3T3 (A, C, E) or human glioblastoma cells T98G (B, D, F) stained with antibodies for γ-tubulin (A, B, red) and α-tubulin (C, D, green). Superpositions of images are shown in E and F. Scale bar, 20 μm.

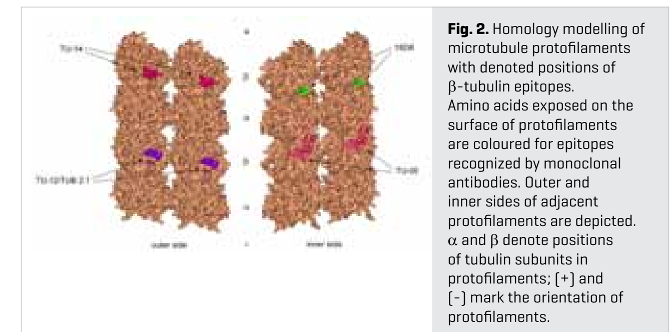

Fig. 2. Homology modelling of microtubule protofilaments with denoted positions of β-tubulin epitopes. Amino acids exposed on the surface of protofilaments are coloured for epitopes recognized by monoclonal antibodies. Outer and inner sides of adjacent protofilaments are depicted. α and β denote positions of tubulin subunits in protofilaments; (+) and (-) mark the orientation of protofilaments.

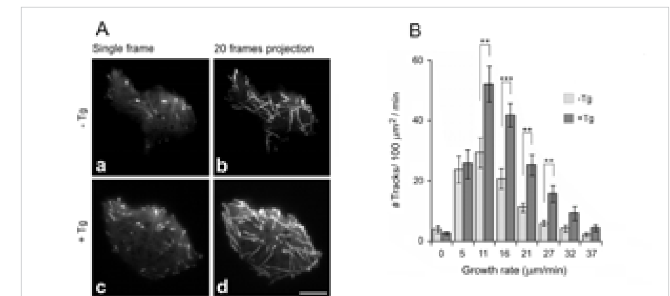

Fig. 3. Activation of mast cells increases the number of growing microtubules in cell periphery as determined by TIRFM time-lapse imaging. (A) Time-lapse imaging of resting (a-b) and thapsigargin (Tg)-activated (c-d) mast cells expressing EB1-GFP that marks growing microtubules. Still images of EB1 (a, c) and tracks of EB1 comets over 20 sec (b, d) in TIRFM. (B) Histogram of microtubule growth rates in cell periphery of resting (-Tg) and thapsigargin-activated (+Tg) cells.

- GA CR, GA204/09/1777 – Analysis of signalling assemblies containing γ-tubulin, 2009-2011, Pavel Dráber
- GA CR, GAP302/10/1701 – Analysis of functional differences between γ-tubulins, 2010-2012, E. Dráberová
- Ministry of Education, Youth and Sports of the Czech Republic, LC545 – Centre of Functional Organization of the Cell, 2005-2011, Pavel Dráber
- AS CR, KAN200520701 – Nano-PCR – ultrasensitive test for detection of specific proteins in body fluids, 2007-2011, Pavel Dráber

1. Hájková Z, Bugajev V, Dráberová E, Vinopal S, Dráberová L, Janáček J, Dráber Pe, Dráber P. STIM1-directed reorganization of microtubules in activated mast cells. **J Immunol** 2011 186(2): 913-923.
2. Dráberová E, Sulimenko V, Sulimenko T, Böhm KJ, Dráber P. Recovery of tubulin functions after freeze-drying in the presence of trehalose. **Anal Biochem 2010** 397(1): 67-72.
3. Blume Y, Yemets A, Sheremet Y, Nyporko A, Sulimenko V, Sulimenko T, Dráber P. Exposure of β-tubulin regions defined by antibodies on an *Arabidopsis thaliana* microtubule protofilament model and in the cells. **BMC Plant Biol 2010** 10(1): 29.
4. Katsetos CD, Dráberová E, Legido A, Dráber P. Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. II. γ-Tubulin. **J Cell Physiol 2009** 221(3): 514-520.
5. Macurek L, Dráberová E, Richterová V, Sulimenko V, Sulimenko T, Dráberová L, Marková V, Dráber P. Regulation of microtubule nucleation from membranes by complexes of membrane-bound γ-tubulin with Fyn kinase and phosphoinositide 3-kinase. **Biochem J 2008** 416(3): 421-430.

From left:
Eduarda Dráberová, PhD / Research Fellow
Zuzana Hájková / Diploma Student
Markéta Černohorská, MSc / PhD Student
Věra Richterová, MSc / Research Assistant
Ladislav Cupák / Technician
Stanislav Vinopal, MSc / PhD Student
Irena Mlchová / Technician
Tetyana Sulimenko, MSc / Research Assistant
Vadym Sulimenko, PhD / Research Fellow
Jan Pačes / Diploma Student
Pavel Dráber, Asoc Prof, PhD / Head of Laboratory

Not on the picture:
Karel Beránek / Diploma Student
Vladimíra Marková, PhD / Research Assistant (maternity leave)
Barbora Hořejší, MSc / PhD Student (maternity leave)