Laboratory of Biology of Cytoskeleton

Modulation of microtubule organization, microtubule proteins, γ-tubulin, signal transduction

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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 TIRF microscopy, live cell imaging and quantification of MT plus end dynamics. Our results demonstrate that Ca2+ plays an important role in the regulation of MT organization in activated mast cells. We have also shown that γ-tubulin, which is assumed to be a typical cytosolic protein, is also present in nucleoli of mammalian interphase cells of diverse cellular origin. Nuclear γ-tubulin associates with tumour suppressor protein C53 and modulates DNA damage G2/M checkpoint activation. We have demonstrated that even though γ-tubulins are differentially expressed during mouse embryogenesis and in adult tissues, they are functionally redundant with respect to their nucleation activity. Finally, we have shown that MT-severing ATPase spastin is overexpresed in glioblastoma multiforme, the most common and deadliest form of primary brain cancers, and that spastin expression might be linked to tumour cell motility, proliferation and invasion.

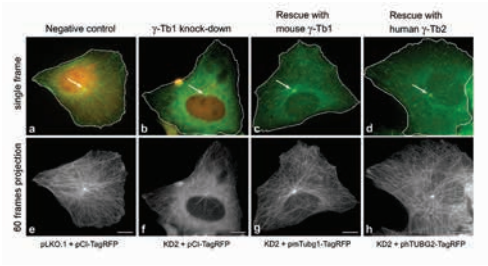

Fig. 1. γ-Tubulin 2 rescues microtubule formation in γ-tubulin 1-depleted cells. Time-lapse imaging of U2OS-EB1 cells for quantitative evaluation of microtubule (+) end dynamics. Cells with depleted γ-tubulin 1 (KD2) expressing either TagRFP (pCI-TagRFP), mouse γ-tubulin 1 (pmTubg1-TagRFP) or human γ-tubulin 2 (phTUBG2-TagRFP). (a-d) Still images of typical cells selected for evaluation. (e-f) Maximum intensity projections of 60 consecutive time-frames from acquired time-lapse sequences. Microtubule track density is rescued in cells expressing exogenous mouse γ-tubulin 1 (g) or exogenous human γ-tubulin 2 (h).

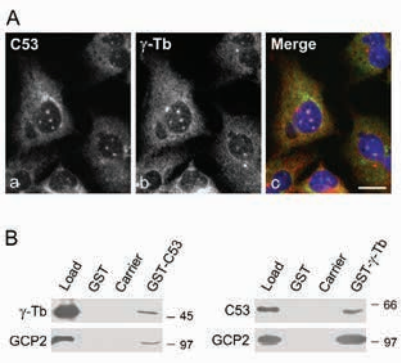

Fig. 2. Association of nucleolar γ-tubulin with tumour suppressor protein C53. (A) Nucleolar localization of C53 and γ-tubulin. Cells were double-label stained with antibodies to C53 (a; green) and γ-tubulin (b; red). DAPI (c; blue). (B) Interaction of γ-tubulin with C53 in GST pull-down assay. Lysates (Load) were incubated with immobilized GST alone (GST), beads used for immobilization (Carrier) or immobilized GST-fusion proteins (GST-C53; GST- γ-tubulin). Blots of bound proteins were probed with antibodies to γ-tubulin (γ-Tb), C53 and γ-tubulin-associated protein GCP2.

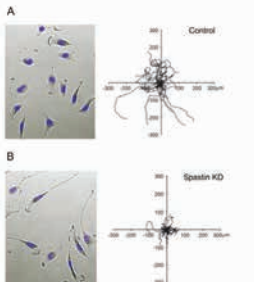

Fig. 3. Effect of spastin depletion on morphology and migration of glioblastoma T98G cells. (A) Control cells. (B) Spastin-depleted cells (spastin KD). Still images from time-lapse imaging; combination of bright field and fluorescence of nuclei. Randomly chosen time-lapse sequences were analysed and all tracks were aligned, with their starting points at coordinate position [0,0].

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- GA CR, GAP302/10/1701 - Analysis of functional differences between γ-tubulins, 2010–2012, E. Dráberová
- GA CR, GPP302/11/P709 - Analysis of microtubular changes during activation of mast cells, 2011–2013, V. Sulimenko
- TA CR, TA01010436 - New generations of DNA aptamers, 2011–2013, Pe. Dráber, Pa. Dráber
- Ministry of Education, Youth and Sports of the Czech Republic, LH12050 LH-KONTAKT II - Regulation of microtubule formation in brain cancer cells, 2012–2015, P. Dráber
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