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. Our current work focuses on understanding the modulation of MT properties by signal transduction molecules, the function of γ-tubulin forms, and on molecular and functional characterization of the regulators of MT nucleation. To address these questions, the techniques of molecular biology, biochemistry and immunology are being used, as well as a variety of microscopic techniques, including TIRF microscopy, SIM, live cell imaging and quantification of MT plus-end dynamics. Our results demonstrate that p21-activated kinase interacting exchange factor (PIX) and G protein-coupled receptor kinase-interacting protein 1 (GIT1) play an important role in MT nucleation. Both proteins can associate with centrosomes of interphase cells. Microtubule regrowth and phenotypic rescue experiments showed that PIX and GIT1 represent, respectively, negative and positive regulators of MT nucleation. Moreover, in mast cells MT nucleation is modulated by Ca2+, which affects γ-tubulin binding properties. We have also shown that both human γ-tubulins are nucleation competent but differ in their properties and expression. Accumulation of γ-tubulin 2 in mature neurons, in the face of predominant γ-tubulin 1 expression in these cells, may reflect additional γ-tubulin 2 function(s) in the neurons. We have demonstrated that ectopic expressions of γ-tubulin complex proteins GCP2 and GCP3 may represent novel markers in the pathobiology of glioblastoma multiforme, the most common and deadliest form of primary brain cancers. Although GCP2 and GCP3 are assumed to be typical cytosolic proteins, they are, similarly as γ-tubulin, also present in nucleoli of glioblastoma cells. Finally, we have introduced new methods for quantification of α-tubulin isotypes and for detection of tau proteins in cerebrospinal fluids.

Fig. 1. Subcellular localization of GFP-tagged PIX and GIT1 in bone-marrow mast cells Cells expressing TagRFP-γ-tubulin and JIP1-GFP or GIT1-GFP were fixed and evaluated in centrosomal region by super-resolution microscopy. (a-c) Localization of γ-tubulin (a) and JIP1 (b). Superposition of images (c, γ-tubulin, red; JIP1, green). (d-f) Localization of γ-tubulin (d) and GIT1 (e). Superposition of Images (f, γ-tubulin, red; GIT1, green). Bar, 2 μm.

Fig. 2. JIP1 and GIT1 proteins affect microtubule nucleation in bone-marrow mast cells The distributions of γ-tubulin fluorescence intensities (arbitrary units, AU) in 1 μm ROI at 1.5 min of microtubule regrowth shown as box plot diagrams. (A) JIP1-depleted cells (mJIP1-KD1 + hJIP1) relative to control cells (Control, pLKO.1). (B) GIT1-depleted cells rescued by hGIT1 (mGIT1-KD1 + hGIT1) relative to control cells (Control, pLKO.1). (C) GIT1-depleted cells rescued by nGIT1 (mGIT1-KD1 + nGIT1) relative to control cells (Control, pLKO.1 + pCDH).

Fig. 3. Discrimination of human γ-tubulins Human γ-tubulin 1 is specifically recognized by anti-peptide antibody TU-30. (A) U2OS cells expressing human FLAG-tagged γ-tubulin 1 (TUBG1-FLAG) or γ-tubulin 2 (TUBG2-FLAG) were immunofluorescence stained for FLAG (green) and γ-tubulin 1 (red, TU-30). DNA was labelled with DAPI (blue). Scale bar, 20 μm. (B) Immunoblot of total cell lysates from cells expressing TagRFP-tagged human γ-tubulin 1 (γ-T1) or γ-tubulin 2 (γ-T2), probed with mouse antibodies to γ-tubulin (TU-30 and TU-31), probed with mouse antibodies to γ-tubulin (TU-30 and TU-31), tagRFP (RRF1) or GAPDH. In control samples, only secondary anti-mouse antibody was applied.
From the left: Irena Michová / Technician, Eduarda Dráberová, PhD / Research Fellow, Jana Štíhlová Bc / Diploma Student, Assoc Prof Pavel Dráber, PhD / Head of Laboratory, Vadym Sulimenko, PhD / Research Fellow, Markéta Černohorská, MSc / PhD Student, Ladislav Cupák Bc / Technician, Tetyana Sulimenko, MSc / Research Assistant, Vladimíra Sládková, MSc / Research Assistant.

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