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Molecular Mechanisms of Microtubule Nucleation and Organization

Komise pro obhajoby doktorských disertací v oboru
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Summary

Microtubules (MTs) formed by αβ-tubulin dimers are highly dynamic structures essential for the spatio-temporal intracellular organization and transport, signal propagation, cell differentiation, motility and division. To perform these roles, MTs create arrangements capable of fast and precise adaptation to various signals. MTs are under the control of many factors regulating their nucleation, stability and dynamics. In this thesis I summarized more than 20 years of my work in the field of MT regulation. The thesis provides a brief overview of our current understanding of MT structure, dynamics and MT regulators. Then it traces our efforts to contribute to a better understanding of the complexity and function of regulatory proteins, mainly γ-tubulins, affecting MT nucleation and organization.

We have proved on various models that γ-tubulin is located not only on centrosomes but also on the other cellular structures, and its distribution changes during differentiation events and cell division. Moreover we have shown, that γ-tubulin is not a universal nucleator of MTs. Although two mammalian γ-tubulin isotypes are highly conserved, we were capable to discriminate them in various cell types. Accumulation of γ-tubulin-2 in mature neurons and neuroblastoma during oxidative stress may signify its prosurvival function, whereas the main function of the dominant γ-tubulin-1 appears to be centered on MT nucleation. Investigation of human *TUBG1* missense mutations revealed that disease-related *TUBG1* variants exert their pathogenicity by affecting MT dynamics. We have shown that γ-tubulin is capable to generate oligomers, forms complexes with αβIII-tubulin dimers, and its binding properties change during the differentiation processes. These findings indicate that, apart from large (γTuRC) and small (γTuSC) complexes, other molecular forms of γ-tubulin exist. γ-Tubulin is post-translationally modified, and these modifications might regulate interactions of γ-tubulin with the other proteins associated with γ-tubulin.

We have found that γ-tubulin can be in complexes with protein tyrosine kinases of Src and Syk families, protein tyrosine phosphatase SHP-1 and phosphatidylinositol-3-kinase (PI3K), which are involved in signal transduction. We proved phosphorylation of γ-tubulin and verified that selective inhibition of Src family kinases reduce the amount of phosphorylated γ-tubulin. Tyrosine kinases and phosphatases therefore could play important role in the regulation of γ-tubulin interactions. We have discovered intrinsic association of γ-tubulin with membranes, and revealed that membrane-bound γ-tubulin complexes promote MT nucleation that is dependent on the activity of Src-family kinases and PI3K.

We have shown that activation of mast cells results in rapid and transient generation of protrusion containing MTs. The formation of protrusions is dependent on the influx of extracellular Ca^{2+} that also affects MT nucleation and γ-tubulin properties. Inhibition of Ca^{2+}-dependent PKC kinases blocks generation of the protrusions. MT nucleation both in mast cells and other cell types is regulated by GIT1/βPIX signaling proteins and PAK1 kinase, which are generally considered to be microfilament regulators. We have found that profilin, controlling actin nucleation, dynamically associates with MTs via formins. This contributes to balancing actin assembly during the cell growth and affects the MT dynamics. Such regulatory role of profilin suggests a close actin-microtubule interrelationship.

Finally, we have found enhanced expression of γ-tubulin in gliomas and medulloblastomas. Moreover, increased levels of βIII-tubulin, γ-tubulin complex proteins (GCP2/GCP3) and microtubule-severing ATPase spastin were characteristic features of highly motile glioblastoma cells. Spastin depletion resulted in inhibition of cellular motility. Both γ-tubulin and GCP2/GCP3 were identified in the nucleus/nucleolus of glioblastoma cells, and overexpression of γ-tubulin or GCP2 antagonized the inhibitory effect of tumor suppressor CDK5RAP3 on the DNA damage G2/M checkpoint activity. These findings suggest that γTuSC dysregulation might be linked to altered transcriptional checkpoint activity or interaction with signaling pathways associated with a malignant phenotype.

Abbreviations

BMMC	Bone marrow-derived mast cell
CAMSAP	Calmodulin-regulated spectrin-associated protein
CDK5RAP3	CDK5 regulatory subunit-associated protein 3 (C53)
CSF	Cerebrospinal fluid
FcεRI	High-affinity IgE receptor
EB	End-binding protein
GBM	Glioblastoma multiforme
GCP	γ-Tubulin complex protein
GIT1	G protein-coupled receptor kinase-interactin g protein 1
MAP	Microtubule-associated protein
MCD	Malformation of brain cortical development
MTOC	Microtubule organizing center
Nano-iPCR	Gold-nanoparticle-based immuno-PCR
PAK1	p21 protein [Cdc42/Rac]-activated kinase 1
PI3K	Phosphatidylinositol-3-kinase
βPIX	p21-activated kinase interacting exchange factor β
PTM	Post-translational modification
SH2	Src homology 2
STIM1	Stromal interacting molecule 1
TBA	Tubulin binding agent
+TIP	Microtubule plus-end tracking protein
γTuC	γ-Tubulin complex
γTuRC	γ-Tubulin ring complex
γTuSC	γ-Tubulin small complex

1. Introduction

Microtubules, together with microfilaments and intermediate filaments, participate in generation of the cytoskeletal filament system. Microtubules are 25 nm cylindrical polymers indispensable for many vital cellular activities such as maintenance of cell shape, division, migration and ordered vesicle transport powered by motor proteins. They are also essential in organizing the spatial distribution of organelles in interphase cells. Microtubules fulfill many of their functions by forming specific assemblies, such as the radial cytoplasmic network, axonemes, centrioles, mitotic/meiotic spindles, and midbodies during cytokinesis (1). The basic building blocks of microtubules are heterodimers of globular α- and β-tubulin subunits, which are non-covalently arranged in a head-to-tail fashion to 13 protofilaments that form a microtubule.

1.1. Microtubule structure and dynamics

In mammals, tubulin heterodimers represent 3-4% of the total protein content in cells, and reach up to 20% in the brain. The secondary and tertiary structures of the α- and β-monomers are essentially identical, as expected from their identity of >40% over the entire sequence of above 445 amino acids of the sequence (2). Each tubulin monomer binds one molecule of GTP, non-exchangeably in the α-subunit (N-site) and exchangeably in the β-subunit (E-site). *In vivo*, the cylindrical and left-handed helical microtubule wall typically comprises 13 parallel protofilaments. Microtubules are thus inherently polar, and contain two structurally distinct ends: a slow-growing minus (-) end, exposing α-tubulin subunits, and a fast-growing plus (+) end, exposing β-tubulin subunits (3). Docking the high-resolution structure of αβ-tubulin dimers (4) into lower-resolution structure of microtubule (5) revealed that tubulin dimers form a B lattice, where the main lateral contacts across protofilaments are between subunits of the same type (i.e., α-α, β-β). However, in microtubule seam lateral contacts are reversed (i.e., α-β, β-α) (6) (**Figure 1**). High resolution of microtubule structures (4.7-5.6 Å) revealed structural transitions in the αβ-tubulin dimer upon GTP hydrolysis (7). The microtubular surface displays a surprisingly large number of binding sites, with numerous proteins binding to the outside surface and a multitude of small ligands binding to the inside of microtubules (8). An important group of ligands that bind to tubulin and change the stability of microtubules are tubulin-binding agents (TBA; tubulin drugs), which have been successfully used in anticancer therapy (9).

Typically in mammalian cells, dynamic microtubules are attached by their (-) ends to microtubule-organizing centers (MTOC), whereas their (+) ends are dynamic and switch between phases of growth and shrinkage. Assembly and disassembly of microtubules is driven by the

binding, hydrolysis and exchange of GTP on the β-tubulin monomer. It is generally accepted that the tip of newly formed microtubule contains a cap of GTP-tubulin (denoted the GTP cap), which has stabilizing properties, whereas the microtubule shaft is composed of GDP-tubulin and is intrinsically unstable. The GTP-cap model explains the dynamic instability of microtubules: in the presence of the cap, a microtubule continues growing, and loss of the cap leads to rapid microtubule shrinkage. This phenomenon is called "dynamic instability of microtubules", an essential feature of microtubules that allows them to search through the cell for targets, such as chromosomal kinetochores, the cell cortex and actin cytoskeleton (10). The growing (+) end often exhibits

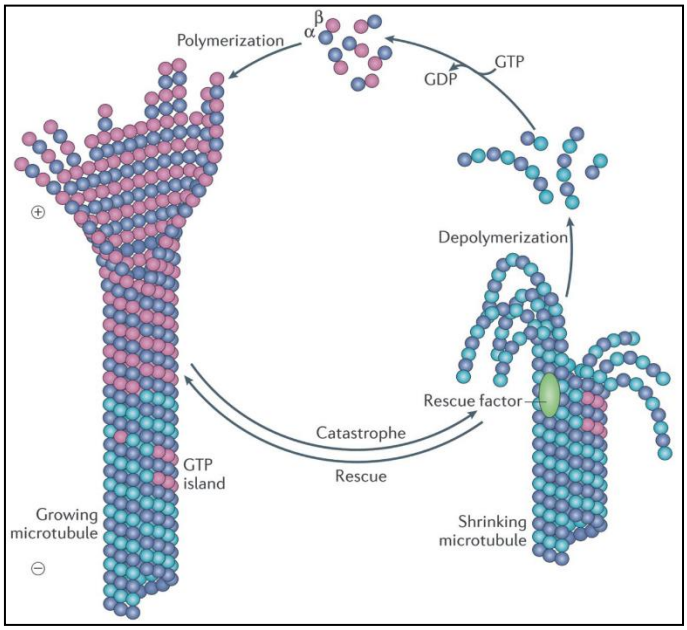

Figure 1. Dynamic instability of microtubules. The cycle of tubulin assembly and disassembly is driven by hydrolysis of GTP bound to β-tubulin. The GTP-bound tubulin dimers are incorporated into the growing microtubule (sheet-like structure at the (+) end). Because the GTP hydrolysis occurs with a delay, the generated GTP cap stabilizes the microtubule tip. The loss of GTP cap leads to a catastrophe and rapid disassembly, resulting in shrinkage of the microtubule. The rescue might be induced by GTP islands in the microtubule lattice or by rescue factors. α-Tubulin is in blue, β-tubulin with GTP is purple and β-tubulin with GDP is in cyan (11).

curved, flattened and tapered sheet-like structures. The shrinking (+) end is denoted by peeling heavily curved protofilaments. The curved protofilament structure of depolymerizing microtubule ends is an intrinsic feature of unpolymerized tubulin, in both the GDP- and the GTP-bound form. Transitions between the curved and the straight conformation of tubulin are important for controlling microtubule dynamics. The mechanisms underlying the transitions between growth and shrinkage (that is, microtubule rescues and catastrophes, respectively; **Figure 1**) are complex and not fully understood (11).

Microtubules that have been growing for a longer time ("older" microtubules) have a higher chance of undergoing a catastrophe. The nature of the catastrophe-promoting events is unknown,

but they might involve accumulation of microtubule-lattice defects or increased tapering of the growing microtubule end (12). Microtubule rescues are understood even less well than catastrophes. Rescues might be induced by local lattice features that can halt microtubule disassembly, such as "GTP islands" of GTP-tubulin that mimic the stabilizing GTP cap (13). It was shown that the lattice defects promote rapid subunit exchange leading to the incorporation of fresh GTP-tubulin and propensity to rescue episodes of shrinkage (14).

1.2. Tubulin isotypes and post-translational modifications

Both α- and β-tubulin consist of isotypes encoded by different genes, and differing in amino acid sequences. Alignment of amino acid sequences of the α- and β-tubulin isotypes revealed that most of the divergence is contained in the last 20 amino acids in unstructured C-terminal tail (15), which lies on the exterior of the microtubule and is the putative binding site for microtubule-associated proteins (MAPs) (16). Differences among isotypes are often highly conserved in evolution, suggesting that they have functional significance. In humans, eight α-tubulin and nine β-tubulin genes were identified (17). Besides, other very different forms of tubulin have been discovered, designated as γ, δ, ε, ζ, η, θ, ι and κ (18). Interestingly, all have been found either in the centrosome, or in the very similar basal body. Some of these tubulins play a significant role in the assembly of these organelles (19). Together with α- and β-subunits, these tubulins constitute the tubulin superfamily. Several of them are widespread among eukaryotes (α, β, γ, δ), while others are more restricted (18, 20).

Various α- and β-tubulin isotypes often differ in their cellular and tissue distribution. Besides, purified isotypes display differing properties including microtubule assembly, conformation, GTPase, dynamics, ability to interact with TBA and MAPs (21). Vertebrate β-tubulin isotypes have fairly distinct tissue distributions (22). Compared to other β-tubulin isotypes, βIII-tubulin possesses certain peculiar properties, which may account for its unique function(s). Unlike the other β-tubulin isotypes, βIII-tubulin lacks oxidation-sensitive residue Cys239, and this permits αβIII-tubulin dimers to assemble in the presence of free radicals (23). Moreover, the presence of Thr429 in βIII-tubulin strongly favors microtubule assembly (24). Finally, despite its restricted and predominantly neuronal cell-type distribution in normal organs and tissues, the βIII isotype is widely expressed in a broad range of human tumors of neuronal and non-neuronal origin (25).

Microtubule specialization is regulated by the "tubulin code", which is generated by expression of different tubulin isotypes and by post-translational modifications (PTMs) of both tubulin subunits. Most tubulin PTMs are generated on the microtubule polymer, and modified tubulins are non-uniformly distributed along microtubules. Strongly modified stable microtubules are

concentrated in specialized organelles, such as centrioles and cilia, or axons of neurons. Well-characterized PTMs include acetylation, detyrosination and tyrosination, Δ2- and Δ3-tubulin, (poly)glutamylation and (poly)glycylation. Except acetylation, these PTMs are found within the C-terminal tails of tubulin subunits. Besides the above-described PTMs, there are other PTMs such as phosphorylation, methylation, polyamination, palmitoylation, arginylation, glycosylation, nitration, ubiquitylation, and sumoylation (26). Such a large number of tubulin PTMs, together with their combinations, points to the possibility of the occurrence of specific microtubule regions that can influence interactions between microtubules and MAPs.

1.3. Regulators of microtubule ends

The dynamic instability of microtubules and the connections between microtubules and cellular structures are spatially and temporally controlled by numerous proteins, which can be broadly grouped into MAPs and molecular motors, the mechanochemical ATPases kinesins and dyneins (microtubule motor proteins), that use microtubules as pathways for intracellular transport. MAPs can be found all along the MT lattice or interact with (+) or (-) ends of microtubules. They can be divided into microtubule-stabilizing MAPs, microtubule-severing proteins, microtubule cross-linking proteins, and microtubule-assembly and disassembly promoters. Microtubule (+) end-tracking proteins (+TIPs), which include structurally and functionally diverse microtubule regulators, are distinguished by their ability to concentrate at growing microtubule ends (27).

End-binding proteins (EBs) are master regulators of +TIP networks, as they recognize growing microtubule ends, and then recruit a range of different factors to these important locations. The EBs localize to growing microtubule ends to form comet-like accumulations (11). The binding of EB1 promotes microtubule maturation by accelerating the transition of GTP- to GDP-tubulin and by promoting lateral protofilament interactions (28). The coordinated recruitment of multiple proteins with distinct activities to microtubule ends allows cells to couple the control of microtubule dynamics to specific cellular sites or signaling events. +TIP networks can guide microtubule growth, control the attachment of microtubule tips to cellular structures, and concentrate molecules for signaling and transport purposes (11).

Proteins associated with free microtubule (-) ends are less understood. The main factor that specifically binds to this microtubule site is γ-tubulin ring complex (γTuRC) (see next Section 1.4.). Recently, members of the calmodulin-regulated spectrin-associated protein family (CAMSAPs) have emerged as regulators of free microtubule (-) ends that function independently of γ-tubulin (29). Their end specificity is mediated by C-terminal CKK domains, which are necessary and sufficient for microtubule (-) end binding and recognize specific tubulin conformations (30).

CAMSAPs stabilizing the (-) ends of non-centrosomal microtubules are important for cell polarity, regulation of neuronal differentiation and definition of spindle organization.

1.4. γ-Tubulin complexes and microtubule nucleation

One of the key components required for microtubule nucleation and stabilization is γ-tubulin (31), a highly conserved and minor member of the tubulin superfamily. There is about 30% identity between γ-tubulin and αβ-tubulin dimers. Whereas multiple gene families encode the α- and β-tubulins, only two functional genes exist in mammalian cells (in humans denoted *TUBG1, TUBG2).* They are located on the same chromosome in tandem, and their coding sequences exhibit high sequence similarity (94% in humans). At the protein level, γ-tubulin-1 and γ-tubulin-2 differ, both in the mouse and human, only by ten amino acids. Whereas γ-tubulin-1 is expressed ubiquitously, γ-tubulin-2 is primarily expressed in the brain (32, 33). γ-Tubulin is post-translationally modified, and phosphorylation at multiple sites and ubiquitylation were reported. In γ-tubulin immunocomplexes, kinases were repeatedly identified, which suggests that kinases might be involved in the regulation of γ-tubulin interactions (34).

The γ-tubulin, together with other proteins, named γ-tubulin complex proteins (GCPs) in humans, assembles into γ-tubulin complexes (γTuCs). The γ-tubulin small complex (γTuSC) is composed of two molecules of γ-tubulin and one copy each of GCP2 and GCP3 (35). It has a form of V-shaped structure with γ-tubulins located on the tips of two arms. The complex is flexible, with a hinge-like motion near the center of the GCP3 arm. The movement about this hinge alters position of γ-tubulin molecules (36). The large oligomeric γ-tubulin-ring complex (γTuRC) comprises γTuSCs and additional GCPs, GCP4-6. In the current γTuRC model, GCP2-6 each bind directly to γ-tubulin and assemble into the conical structure that contains 13 γ-tubulin molecules and effectively nucleates microtubules. Both TuSCs and γTuSC-like assemblies are used for generation of γTuRCs. Hybrid γTuSCs are created by replacement of one of the GCP2-3 with one of GCP4-6. Novel γTuSCs have both GCP2 and GCP3 changed for any combination from GCP4-6 proteins, and half γTuSCs can be composed of a single γ-tubulin and one molecule of GCP4-6 (**Figure 2**). The flexibility of the GCP3 hinge is essential for closing γTuRC in a conformation compatible with the geometry of the microtubule. In this template-based nucleation model, γTuRC provides the platform for the assembly of αβ-tubulin heterodimers. As GCPs are phosphorylated (37), their phosphorylation can regulate conformational changes that might be required for γTuRC activation (38). Organization of proteins in γ-tubulin complexes is depicted in **Figure 2**. Nucleation from templates is in itself a kinetically unfavorable process that is limited by the formation of a (+) end capable of persistent growth. Factors that actively support formation of an actively growing (+) end

such as anti-catastrophe protein TPX2 or microtubule polymerase XMAP215 (+TIP) stimulate the template nucleation. On the other hand, GTP hydrolysis inhibits microtubule nucleation by destabilizing the nascent (+) ends (39).

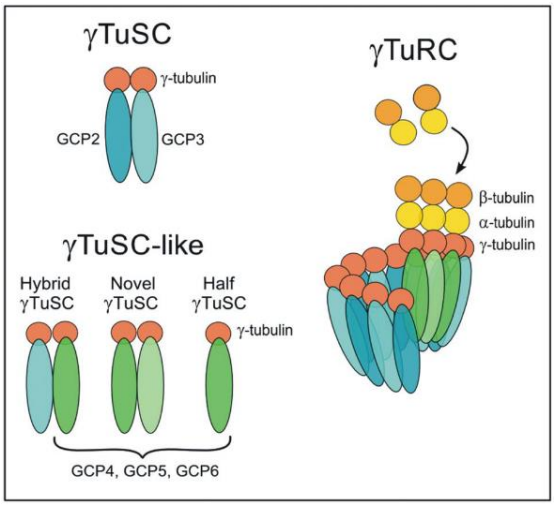

Figure 2. γ-Tubulin complexes and model of γTuRC assembly. γTuSC is composed of GCP2 and GCP3 and two molecules of γ-tubulin. γTuSC-like structures can be formed by replacement of GCP2 or GCP3 with GCP4, GCP5 or GCP6. γTuRC is generated by association of γTuSCs and γTuSCs-like complexes. In the template nucleation model, γ-tubulins in the γTuRC serve as a platform for binding of αβ-tubulin heterodimers (34).

While soluble γ-tubulin complexes are highly abundant in the cytoplasm, their nucleating activity is limited to specific cellular locations. Various associated proteins are involved in the regulation of γ-tubulin complexes. These proteins target γTuCs to specific sites or activate their nucleation activity. Anchoring proteins play an important role in microtubule nucleation because they affect recruitment of complexes to specific sites. Finally, modulating proteins can also affect nucleation, but their role is less obvious. Microtubule nucleation in mammalian cells can occur from microtubule organizing centers (MTOCs) as centrosomes or from non-centrosomal MTOCs as Golgi apparatus, nuclear envelope, chromatin and kinetochores, surface of other microtubules and plasma membrane-associated sites (34).

Centrosomes are composed of two barrel-shaped orthogonally arranged centrioles wrapped in highly structured multicomponent pericentriolar matrix (PCM) responsible for microtubule nucleation (40). Many proteins participate in targeting γTuCs to the centrosomes; the best characterized of them are CDK5RAP2, NEDD1, Mozart1, and Mozart2. Anchoring proteins such as AKAP450, pericentrin, ninein, and Cep192 are important for localization of γTuRC to the centrosomes, as they are incorporated in PCM (41). After recruitment to the centrosome, γTuRC is activated by switching from the open to the closed form of γTuRC (42). In this process, CDK5RAP2 participates by activating γTuRC-mediated nucleation ~ 7-fold, while another

nucleation activator, kinase NME7, increases the nucleation capacity of γTuRC ~ 2.5-fold (43). The regulatory role in centrosomal microtubule nucleation can also be played by signaling proteins. It was reported that various kinases and proteins of the GIT/PIX/PAK signaling complex modulate the microtubule nucleation (34).

1.5. γ-Tubulin functions beyond microtubule nucleation

Increasing evidence indicates that γ-tubulin has functions beyond the microtubule nucleation. There have been repeated reports that mutations or deficiencies in γ-tubulin or GCPs alter (+) end microtubule dynamics. A microtubule nucleation-independent role for γ-tubulin complexes was also described in the control of the spindle assembly checkpoint (SAC) and mitotic exit, as well as in cell cycle progression in interphase (44). There are also indications that nuclear γ-tubulin modulates the activity of E2F transcription factors, which control expression of several genes that are necessary for DNA replication and centrosome duplication (45). Retinoblastoma RB1 protein and γ-tubulin negatively regulate each other's expression. A high level of γ-tubulin was detected in cells with impaired RB1, while RB1 was overexpressed in γ-tubulin mutants (46).

γ-Tubulin has the intrinsic property to generate oligomers *in vitro*. It was reported that in cells γ-tubulin forms fine fibers both in the cytosol and nuclei (47). What is the function of such fibrillar structures is unknown, but it was suggested that they can play scaffolding or sequestration functions. They could also be involved in mechanotransduction as they interact with the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex (48, 49). γ-Tubulin is also a component of mitochondria. In neuronal cells, expression of γ-tubulin-2 increases under mitochondria-derived oxidative stress, signifying a prosurvival function of γ-tubulin-2 under stress (50).

Although the functional significance is not yet clear, γ-tubulin is associated with tumor suppressor proteins involved in DNA damage checkpoints and DNA repair. It binds to Rad51 in the nuclei in response to DNA damage (51), and is included in complexes with ATR (52), BRCA1 (53) and CDK5RAP3 (54). Tubulin is overexpressed in a variety of cancer cells, including some multiple myelomas, non-small cell lung cancers, breast cancer, gliomas and medulloblastoma (55). γ-Tubulin thus can play an important role in tumorigenesis. In other diseases, including Parkinson's disease and dementia with Lewy bodies, γ-tubulin has been localized to aggresomes (56). Interestingly, mutations in *TUBG1* are associated with malformations of cortical development (MCD) linked to epilepsy and intellectual delay (57, 58). Disease-related *TUBG1* variants exert their pathogenicity by affecting microtubule dynamics and by disrupting neuronal migration (59).

2. Aims

The research work presented in this thesis focused on understanding of microtubule nucleation and organization. The main task was deciphering the function and regulation of γ-tubulins and their interactors. Specific topics were as follows:

1. Determination of the subcellular localization of γ-tubulins during the cell cycle, differentiation and development;
2. Identification and characterization of proteins interacting with nucleation complexes;
3. Biochemical characterization of γ-tubulin isotypes, their molecular forms and elucidation of their functions;
4. Deciphering the role of signaling proteins in the regulation of microtubule nucleation and microtubule organization;
5. Understanding the role of microtubule nucleating proteins in cancer cells.

3. Methods

This section summarizes experimental models and provides a short description of unique methods as well as the results from methodological publications. Bold numbers in brackets refer to original publications in Section 9.

3. 1. Experimental models

Although major building components of microtubules are highly conserved in all eukaryotic cells, microtubule properties can be different in various cellular models, which can also employ diverse mechanisms how microtubules are nucleated and anchored in MTOCs. The majority of experiments were performed in mammalian tissue culture cells that have highly dynamic microtubules emanating preferentially from centrosomes. We also used axonemal protozoa that possess highly stable microtubules. To study acentrosomal nucleation of microtubules, we used plant cells, which have a higher amount of γ-tubulin when compared with animal cells. Mature chicken erythrocytes represented an animal model system without MTOCs. To study dysregulation of microtubule proteins in the brain and different tumor types, we used corresponding tissues.

3.2. Monoclonal antibodies

Well-characterized monoclonal antibodies, prepared in the Laboratory of Biology of Cytoskeleton IMG CAS (https://dbc.img.cas.cz/antibodies.html), were applied for immunochemical,

immunocytochemical and immunohistochemical methods used in the presented publications. The first monoclonal antibodies to tubulin, including our antibody TU-01 (60), were published in 1982. Construction of the hybridoma, producing antibody TU-01, was one of the first successful applications of this biotechnology in Czechoslovakia. From the Laboratory originate the first published monoclonal antibodies to γ-tubulin (**9.2**) and to γ-tubulin complex protein 2 (**9.29**). The antibody TU-20 is specific for βIII-tubulin only, which is mainly expressed in neuronal cells (**9.4**). Finally, the antibody TU-30 recognizes an epitope that is present in human γ-tubulin-1 but not on human γ-tubulin-2. This allowed the first immunological discrimination of human γ-tubulin isotypes (**9.33**).

3.3. Immunochemical methods

One of essential steps in the hybridoma technology is adequate storage of monoclonal antibodies. Mouse monoclonal antibodies, in the form of spent tissue culture supernatants, ascitic fluids, or purified antibodies are usually stored as small aliquots at -20°C or -70°C. Alternatively, they are stored at 2-4°C with added preservatives, or they are freeze-dried. Freeze-thaw cycles are potentially damaging, particularly to monoclonal antibodies of the IgM class, which are also prone to aggregation after prolonged storage at 4°C. As IgM is less robust than IgG, many IgM monoclonal antibodies are irreversibly denatured by freeze-drying (61). We described the use of disaccharide trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) for stabilization of mouse monoclonal IgM antibodies during freeze-drying and prolonged storage at elevated temperatures. Antibodies directed against various types of antigens effectively recovered their binding efficiency after dehydration. This permits convenient long-term storage of large quantities of antibodies, facilitates their transport at ambient temperature, and simplifies construction of pre-aliquoted kits based on such antibodies (**9.1**). The maintenance of the proper conformation of antibodies during freeze-drying in the presence of trehalose could be explained by the water replacement hypothesis, assuming that sugar molecules substitute for water from the protein hydration shell (62).

The widespread use of the hybridoma technology for production of specific monoclonal antibodies has created the need for simple, rapid, specific and sensitive methods for quantification of the mouse antibody production in hybridoma culture supernatants. We have developed a cost-effective method for rapid quantification of mouse immunoglobulins in hybridoma culture media. The method consists of spotting 1-μl samples onto a nitrocellulose membrane and staining the dots with anti-mouse antibodies conjugated with horseradish peroxidase. Binding is revealed by incubation with a sensitive chemiluminescence reagent. Quantitation is achieved by densitometric comparison with standard curves produced by purified monoclonal antibodies and takes about 1 h

in total. Good linearity between the staining intensity and the amount of immobilized immunoglobulins was observed over the range of 0.05–5 ng/spot (**9.13**).

3.4. Microtubule proteins

As tubulin is the main soluble protein of neuronal cells, microtubule proteins were mainly purified from porcine brain by repeated temperature-dependent cycles of polymerization and depolymerization (63). The remaining MAPs were removed by chromatography on phosphocellulose (64). To get the assembly-competent tubulin, phosphocellulose purified tubulin was recycled by sodium glutamate to stimulate tubulin polymerization (65). Since tubulin is a thermolabile molecule that converts to the non-polymerizing state within several hours on ice (63), it is important to assure the proper storage that preserves its polymerization capacity. We have found that inclusion of trehalose as a stabilizer during freeze-drying preserves the biological activities of tubulin and allows its long-term storage at ambient temperature (66). This enables to standardize assays for screening of microtubule-active compounds (95).

Microtubule-associated protein tau is an important biomarker, as increased amounts of tau protein in cerebrospinal fluid (CSF) indicate Alzheimer's and other neurodegenerative diseases. ELISAs have been mostly used for quantification of tau in CSF. However, these assays do not possess sufficient sensitivity and dynamic range. We have prepared gold nanoparticles functionalized with tau-specific monoclonal antibody and oligonucleotide template, which in combination with capture anti-tau antibody could be used for immuno-polymerase chain reaction (Nano-iPCR) to quantify the tau protein in CSF. For comparison, the tau protein was also quantified by ELISA using the same tau-specific antibody set and biotinyl-tyramide detection. Biotinyl-tyramide and Nano-iPCR amplification allow, respectively, detection of tau protein at concentrations 140 pg/ml and 5 pg/ml. The data indicate that Nano-iPCR is superior in sensitivity and detection range to ELISA in the tau protein detection. The commercial availability of monoclonal antibodies suitable for tau protein detection by Nano-iPCR and the easy preparation of functionalized gold nanoparticles reduce the expenses for tau protein quantification at least 10 times when compared to assays based on commercial kits (**9.27**).

3. 5. Electrophoretic methods and methods for microtubule nucleation and dynamics

Based on different electrophoretic properties of βIII-tubulin (67), we were capable to discriminate this isotype from the other tubulin isotypes (**9.4**). We were the first to show that the electrophoretic properties of γ-tubulin isotypes, similarly as αβ-tubulin dimers, are dependent on the purity of SDS, and that γ-tubulin is capable to form oligomers under the conditions of native electrophoresis

(**9.10**). Using 2D-PAGE, we separated highly similar γ-tubulin-1 and γ-tubulin-2 isotypes in mouse (**9.24**) and human (**9.33**) cultured cell lines and tissues.

To evaluate microtubule nucleation from membranes, we have modified the microtubule spin-down assay. The washed membranes were sonicated and incubated with purified tubulin in the presence of GTP. After fixation with glutaraldehyde, the membranes were centrifuged through a glycerol cushion on to glass coverslips, postfixed in cold methanol, and used for immunostaining (**9.19**). Microtubule regrowth from centrosomes was followed in a nocodazole washout experiment. Cells growing on coverslips were treated with nocodazole to depolymerize microtubules. The cells were then washed with cold medium to remove the drug, transferred to new medium at higher temperature, and microtubule regrowth was allowed for 1-3 min. After fixation, samples were stained for α-tubulin and γ-tubulin. The measurement of α-tubulin immunofluorescence close to the centrosome reflected the nucleation capacity of centrosomes (68). Alternatively, microtubule nucleation was studied using time-lapse imaging of cells expressing tagged EB1. In this case, newly nucleated microtubules were detected by tracking EB1 comets (98) emanating from the centrosomes (**9.30**).

4. Results

Results presented in the selected collection of publications were obtained during my more than twenty-year-long research activity in the microtubule field. Main results and corresponding short commentaries are presented in this section. Bold numbers in brackets refer to original publications in Section 9.

4.1. γ-Tubulin and its complexes

γ-Tubulin was originally reported as a minor member of the tubulin superfamily participating in the nucleation of microtubules (69). However, the role of γ-tubulin is not only limited to the microtubule nucleation. In recent years, additional functions for γ-tubulin were reported (44). γ-Tubulin-dependent nucleation of microtubules occurs both from canonical MTOCs, such as spindle pole bodies and centrosomes, and additional sites such as the Golgi apparatus, nuclear envelope, plasma membrane-associated sites, chromatin, and surface of pre-existing microtubules. Despite many advances in defining the structure of γ-tubulin complexes and characterization of γTuRC interacting factors, the regulatory mechanisms of microtubule nucleation are not fully understood (**9.32**).

Using anti-peptide monoclonal antibodies to the C-terminal region of γ-tubulin (γ434-449) prepared in the laboratory, we demonstrated that γ-tubulin is associated not only with centrosomes and spindle poles, but also with half-spindles and midbodies in mitotic cells of various origins. In nocodazole washout experiments γ-tubulin was found in multiple nucleation centers. When mitotic cells were cultivated in the presence of taxol, mitotic spindles were disrupted and multiple microtubule asters were generated, with (-) ends of microtubules directed to the aster centers (70). The number of taxol-induced microtubule asters exceeded the number of γ-tubulin-positive spots. These results demonstrated, for the first time, that γ-tubulin is not a universal nucleator of microtubules and can be replaced by other proteins associated with (-) ends of microtubules (**9.2**). A family of CAMSAPs, which associate and regulate the (-) ends of microtubules, were discovered later on (11).

In the course of differentiation of nucleated erythroid cells, highly dynamic microtubules originating from MTOCs are converted into a stable marginal band of microtubules and MTOCs disappear. How microtubules are nucleated during this transition is unclear. We have found that γ-tubulin is present in MTOCs, mitotic spindles, and marginal bands in early stages of erythroid cell differentiation. The association of γ-tubulin with the marginal band was observed even in cells without MTOCs. In postnatal peripheral erythrocytes, γ-tubulin was present only in soluble form and formed complexes with tubulin dimers. After cold-induced depolymerisation, γ-tubulin in erythroid cells formed large clusters that were not observed in matured cells, and re-growth experiments demonstrated that γ-tubulin was not present in distinct nucleation structures at the cell periphery. The presented data demonstrated that γ-tubulin is a substrate for developmentally regulated PTMs and that the binding properties of γ-tubulin or its complexes change during the differentiation events (**9.9**).

We also detected PTM of γ-tubulin in microtubule proteins prepared from the brain by repeated cycles of polymerization and depolymerization. We proposed that PTMs play an important role in the modulation of γ-tubulin interactions with associated proteins or tubulin dimers. We were the first to report that two γ-tubulin isoforms exist in the brain, which persist in differently sized complexes and copolymerize with tubulin dimers. Moreover we have found that under non-denaturing conditions γ-tubulin is capable to form oligomers. These findings indicated that apart from well-described large and small γ-tubulin complexes, γ-tubulin could exist in additional molecular forms (**9.10**).

Mammalian γ-tubulins are encoded by two closely related genes (32), and it was proposed that γ-tubulin-1 represents ubiquitous γ-tubulin, while brain γ-tubulin-2 may have some specific functions, and cannot substitute for γ-tubulin-1 (33). To gain a deeper insight into the potential

functional differences of mammalian γ-tubulins, we examined the subcellular distribution of γ-tubulin-2 in cultured cells, its interactions with GCPs, capability to nucleate microtubules and substitute for γ-tubulin-1. We have documented that mouse γ-tubulin-2 localizes to centrosomes, interacts with GCP2 and GCP4, and nucleates microtubules. Moreover, we showed that *Tubg1* and *Tubg2* genes are differentially transcribed during mouse early embryogenesis, with *Tubg2* transcription being progressively downregulated. We proposed that even though γ-tubulins are differentially expressed during mouse early embryogenesis and in adult tissues, they are functionally redundant concerning their nucleation activity (**9.24**).

The studies on the functions of γ-tubulin isotypes were hampered by the unavailability of antibodies that are capable to distinguish between highly conserved γ-tubulin-1 and γ-tubulin-2. We provided evidence that discrimination between human γ-tubulin isotypes is possible, based on their different electrophoretic properties and immunoreactivity with our monoclonal antibodies. Specifically, antibody TU-30 recognizes a unique epitope on human γ-tubulin-1. We also demonstrated enhanced expression of γ-tubulin-2 during neuronal differentiation of human neuroblastoma cells in the face of unchanged levels of γ-tubulin-1. We have found that despite significant accumulation of γ-tubulin-2 in the adult human brain, γ-tubulin-1 is the dominant isotype and is constitutively expressed in mature neurons. We were also the first to show that enhanced expression of γ-tubulin-2 in neuroblastoma cells was triggered by oxidative stress induced by mitochondrial inhibitors, and that γ-tubulins associate with mitochondria. These data indicate that in the face of predominant γ-tubulin-1 expression, the accumulation of γ-tubulin-2 in mature neurons and neuroblastoma cells during oxidative stress may denote a prosurvival role of γ-tubulin-2 in neurons (**9.33**). The independent confirmation of mitochondrial γ-tubulin was provided later on (71).

Over the past decade, genetic studies have identified numerous causative mutations in centrosomal proteins in subjects with MCDs (72). We investigated the consequences of four human MCD-related *TUBG1* variants (Tyr92Cys, Ser259Leu, Thr331Pro, and Leu387Pro) on the cortical development by using *in-utero* electroporation and a knock-in *Tubg1*$^{Y92C/+}$ mouse model. We showed that pathogenic *TUBG1* variants affected neuronal positioning by disrupting neuronal migration without a major effect on progenitor proliferation. Our results suggested that disease-related *TUBG1* variants exert their pathogenicity by affecting microtubule dynamics rather than centrosomal positioning or nucleation ability. Furthermore, *Tubg1*$^{Y92C/+}$ animals showed neuroanatomical and behavioral defects and increased epileptic cortical activity. We demonstrated that *Tubg1*$^{Y92C/+}$ mice partially mimicked the human phenotype, and therefore represent a relevant model for further investigations of the physiopathology of cortical malformations (**9.37**).

Protozoa *Giardia intestinalis*, a bi-nucleated a mitochondrial flagellate, possesses a complex cytoskeleton based on several microtubular systems. Although it possess eight flagella, γ-tubulin was detected only in four basal bodies, which were formed *de novo* during cell division. γ-Tubulin was not detected in basal body regions of the flagella that persist during the division. These findings suggest that γ-tubulin is not essential for stabilization of (-) ends of microtubules in permanent flagella. As *Girardia* has been classified among ancient lineages of eukaryotic organisms (73), γ-tubulin is a ubiquitous member of the tubulin superfamily through the evolutionary history of eukaryotes (**9.8**). Complex distribution of γ-tubulin was found in *Leishmania.* It was detected in the basal bodies, posterior pole of the cell, in the flagellum, and in the subpellicular microtubules. Immunogold electron microscopy of thin sections of isolated flagella showed that γ-tubulin was associated with the paraflagellar rod that runs adjacent to the axonemal microtubules. Moreover, multiple charge variants of γ-tubulin were detected in the flagella. Interestingly, under different extraction conditions, γ-tubulin in *Leishmania* was found only in insoluble cytoskeletal fractions, in contrast to tubulin dimers that were found both in the cytosol and in the cytoskeletal pool. The unique subcellular distribution and properties of γ-tubulin indicate that γ-tubulin in *Leishmania* could have additional function(s) besides microtubule nucleation (**9.12**).

Acentriolar plant cells represent a useful model for studying microtubule organization as they form multiple microtubule structures during the cell cycle. Some of them are typical only of plants. Although acentriolar plant cells do not have well defined MTOCs, γ-tubulin is associated with all microtubule assemblies (74). We have shown, using anti-mitotic drugs and isolated metaphase chromosomes, that γ-tubulin in plant cells is associated with the kinetochore/centromeric region of chromosomes. γ-Tubulin could therefore directly or indirectly modulate/stabilize the interaction of microtubules with kinetochores, and help to organize the mitotic apparatus via chromosomes (**9.3**). A similar association of γ-tubulin with the kinetochore region was observed after pre-treatment of plant cells with specific inhibitors of cyclin-dependent kinases, purine analogs bohemine and roscovitine. In contrast to the regular bipolar spindle in untreated cells, in drug-treated metaphase cells, abnormally short kinetochore microtubule fibers were detected, and the chromosomes were arranged in a circle, with kinetochores pointing inwards. γ-Tubulin was found in the centers of the circular chromosome configuration in close vicinity to the kinetochores (**9.5**). Later on, it was suggested that after chromosomal RanGTP-dependent microtubule nucleation, short microtubules can be stabilized by connecting their (+) ends to kinetochores and then amplified through microtubule nucleation on preformed microtubules by the augmin-dependent pathway (75).

To rule out that γ-tubulin is relocated to the kinetochore region due to anti-mitotic drugs, which were are used to accumulate metaphase chromosomes, analysis of γ-tubulin distribution was

performed in untreated cells. We have found that γ-tubulin in premitotic nuclei is located in the regions corresponding to pre-kinetochores. It was, however, also found in other nuclear sites. γ-Tubulin in the nuclei is associated with chromatin, as it was released after DNAse treatment. The amount of nuclear γ-tubulin increased in G2 phase of the cell cycle. Moreover, we showed that in plant cells, soluble cytoplasmic γ-tubulin is also present. When compared with animal cells, a substantially higher amount of cytosolic γ-tubulin was found in the plant cells. These results indicate that subcellular compartmentalization of γ-tubulin is an important factor in the organization of specific plant microtubule structures and acentriolar mitotic spindle (**9.7**).

Soluble plant γ-tubulin exists in the form of heterogeneous complexes. In contrast to animal cells, plant high-molecular-weight γ-tubulin complexes are resistant to high ionic strength. Complexes higher than 1 MDa are associated with membranes and nucleate microtubules, while low-molecular-weight complexes do not promote microtubule nucleation. Soluble γ-tubulin interacts with αβ-tubulin dimers and *in vitro* binds to plant microtubules along their whole length. We proposed that the presence of γ-tubulin in protein complexes of various sizes, different properties, and subcellular locations, as well as its interaction with αβ-tubulin heterodimers and the association of γ-tubulin complexes or oligomers with microtubules could reflect the specific needs of the plant cells (**9.11**).

The high abundance of γ-tubulin in *Arabidopsis* cells facilitated purification and characterization of the large molecular species of γ-tubulin. Their analysis by various microscopic techniques revealed the presence of linear filaments with a double protofilament substructure, filament bundles and aggregates. Filament formation from highly purified γ-tubulin free of GCPs was demonstrated for both plant and human γ-tubulin. Super-resolution microscopy of *Arabidopsis* cells revealed fine, short γ-tubulin fibrillar structures enriched on mitotic microtubular arrays that accumulated at the poles of spindles, outer nuclear envelope, and were also present in the nuclei. Our findings that γ-tubulin preserves the capability of prokaryotic tubulins to self-organize into filaments assembling by lateral interaction into bundles suggest that besides microtubule nucleation, γ-tubulin may also have scaffolding or sequestration functions (**9.34**). At the same time, an intriguing report describing filaments formed by large γ-tubulin complexes and pericentrin in animal cells appeared (76).

4.2. Regulation of microtubule nucleation and organization

Mast cells play a pivotal role in innate immunity, allergy, and inflammation. On their surfaces, they express receptors with a high affinity for IgE (FcεRIs). Aggregation of FcεRIs by multivalent antigen-IgE complexes leads to activation of signaling pathways resulting in the release of Ca^{2+}

from internal stores and subsequent activation of store-operated Ca^{2+} entry (SOCE). The influx of free Ca^{2+} is important for replenishment of Ca^{2+} in ER, but also works as a second messenger for further signaling. Activation events result in the release of preformed granule mediators and *de novo* synthesis and secretion of bioactive compounds. Changes in the cell morphology and reorganization of microfilaments and microtubules have been reported during these processes. Activated mast cells therefore represent useful model systems for studies directed to understanding the signaling pathways responsible for rapid and transient changes in microtubules (**9.26**).

During the analysis of soluble γ-tubulin pools in rat basophilic leukemia cells (RBL), which can be activated by crosslinking of FcεRIs, we found association of γ-tubulin with tyrosine-phosphorylated proteins in the activated cells. Between the associated proteins, we identified protein tyrosine kinase Lyn, which belongs to the Src family protein tyrosine kinases that are essential for propagation of signals in the early stages of cell activation. Pre-treatment of cells with a Src family inhibitor resulted in reduction of tyrosine phosphorylation in γ-tubulin complexes. These findings indicated, for the first time, that Src family kinases could regulate γ-tubulin interactions (**9.6**).

To decide whether the Lyn kinase is indispensable for the formation of such complexes, we analyzed interactions of γ-tubulin with kinases and their substrates in resting and activated bone marrow-derived mast cells (BMMCs) isolated from wild-type or Lyn-deficient mice. We have shown that microtubule nucleation is attained even in the absence of the Lyn kinase. We found that γ-tubulin in $Lyn^{-/-}$ BMMCs formed complexes with protein tyrosine kinases Fyn and Syk belonging to the Src and Syk/Zap family, respectively. In this study, we also showed that the activation of BMMCs by FcεRI aggregation leads to rapid but transient polymerization of microtubules and their overall reorganization in activated cells. We proposed that Fyn and Syk kinases are involved in the regulation of the binding properties of γ-tubulin or its associated proteins, and thus modulate the microtubule nucleation in activated mast cells (**9.16**).

When BMMCs were attached to fibronectin and then activated by various means, immunostaining for αβ-tubulin dimer revealed a clear difference in the microtubule distribution in the resting and activated cells. While resting cells had rounded morphology, and microtubules at the cell periphery ran predominantly alongside the plasma membrane, activated cells displayed multiple protrusions containing microtubules (microtubule protrusions). Formation of these protrusions was transient and depended on the influx of extracellular Ca^{2+} and presence of stromal interaction molecule 1 (STIM1), sensor of Ca^{2+} and key regulator of SOCE. Changes in the cytosolic Ca^{2+} concentration also affected microtubule (+) end dynamics. In accordance with the inhibition of antigen-induced Ca^{2+} response and decreased formation of microtubule protrusions in

BMMCs with reduced STIM1, the cells also exhibited impaired chemotactic response to antigen. These findings revealed a tight crosstalk between the microtubular network and Ca^{2+} signaling machinery in the course of mast cell activation (**9.21**). The importance of Ca^{2+} influx on microtubule formation (77) as well as generation of microtubule protrusions in activated mast cells (78) were thereafter independently confirmed.

As in antigen- or pervanadate-activated BMMCs γ-tubulin interacted with a similar set of tyrosine-phosphorylated proteins, we tried to identify these proteins. For this we applied large-scale immunoprecipitations with anti-peptide monoclonal antibody to γ-tubulin and extracts from activated cells. Bound proteins were eluted by the peptide, and tyrosine-phosphoryated proteins were thereafter concentrated by binding to purified Src homology 2 (SH2) domain and identified by mass spectrometry. We found p21-activated kinase (PAK) interacting exchange factor β (βPIX) and G protein-coupled receptor kinase-interacting protein (GIT) 1 as signaling proteins that interacted with γ-tubulin and associated with centrosomes. Interestingly, the enhanced level of free cytosolic Ca^{2+} affects the γ-tubulin properties and stimulates association of GIT1 and GCPs with γ-tubulin. Depletion of βPIX stimulated microtubule nucleation, whereas depletion of GIT1 led to its inhibition. Moreover, microtubule nucleation was also affected by the Ca^{2+} level. This study provided, for the first time, a possible mechanism for the concerted action of tyrosine kinases, GIT1/βPIX proteins, and Ca^{2+} in the propagation of signals leading to the regulation of microtubule nucleation in activated mast cells. Presumably, this action may be involved in the regulation of such important processes in mast cell physiology as antigen-induced degranulation and chemotaxis (**9.28**).

The tyrosine phosphorylation of numerous substrates is only transient and returns to baseline levels several minutes after receptor triggering in BMMCs. It is well accepted that protein tyrosine kinases are essential during mast cell signaling, but the exact function of protein tyrosine phosphatases is less understood (79). We identified SH2 domain-containing protein tyrosine phosphatase 1 (SHP-1), forming complexes with γ-tubulin complex proteins, as a negative regulator of microtubule nucleation from the centrosomes of BMMCs. The regulation is due to the changes in γ-tubulin accumulation. During antigen-induced activation, SHP-1 modulates activity of the Syk kinase, which is located to centrosomes, and affects the organization of microtubules. Deletion of SHP-1 results in a substantial increase of microtubule protrusions in the course of specific activation via FcεRI aggregation. Our data suggested a novel mechanism for attenuation of microtubule formation in later stages of mast cell activation (**9.36**).

Besides protein tyrosine kinases and phosphatases, other signaling molecules might modulate organization of microtubules during the activation of BMMCs. We have found that miltefosine

(hexadecylphosphocholine), a new candidate for treatment of mast cell-driven diseases (80), inhibited reorganization of microtubules, degranulation and antigen-induced chemotaxis in the activated cells. While aggregation and tyrosine phosphorylation of IgE receptors were suppressed in activated cells pre-treated with miltefosine, the Ca^{2+} influx was not affected. Tagged-miltefosine rapidly localized into the cell interior, and inhibited movement of some intracellular granules. Miltefosine inhibited Ca^{2+}- and diacylglycerol-regulated conventional protein kinase C (cPKC) isoforms that are important for mast cell degranulation. Collectively, our data revealed that miltefosine modulates mast cells at multiple sites. This alters the intracellular signaling pathway(s) directed to microtubules, degranulation, and migration (**9.35**).

The findings on the regulatory role of tyrosine kinases in the modulation of γ-tubulin complexes were also elaborated on the model of neuronal differentiation of P19 murine embryonal carcinoma cells treated with *all-trans*-retinoic acid. In the course of this process, substantial changes in the cell morphology and organization of microtubules are accompanied by increased expression of non-receptor Src family kinases Src and Fyn, increased level of proteins phosphorylated on tyrosine, and changes in lipid composition. We have found Src-family dependent phosphorylation of γ-tubulin and phosphotyrosine-dependent binding of γ-tubulin complexes to SH2 domains of kinases. These findings indicated that the activity of γ-tubulin complexes could be regulated by phosphorylation. Tyrosine phosphorylation of γ-tubulin on phylogenetically conserved Y445 affects microtubule organization (81). The combined data suggested that Src family kinases might have an important role in the regulation of γ-tubulin interaction(s) with tubulin dimers or other proteins during neurogenesis (**9.14**).

Further study revealed that in differentiated P19 cells, γ-tubulin is equally distributed to both soluble and membrane fractions, while αβ-tubulin dimers prevail in the cytosol. In differentiated cells, γ-tubulin stained distinct dots in neurite projections, and the fraction of γ-tubulin associated with detergent-resistant membranes. Membrane-bound *γ*-tubulin formed complexes with the Fyn kinase, p85α regulatory subunit of phosphoinositide 3-kinase (PI3K), and nucleated microtubules. Inhibition of Src family kinases or PI3K blocked the nucleation activity of the membrane-bound γ-tubulin complexes. γ-Tubulin interacted directly with the C-terminal SH2 domain of p85*α*, outside the tyrosine-phosphorylated protein-recognition site. The combined results suggested that Fyn and PI3K might take part in the modulation of membrane-associated γ-tubulin activities during non-centrosomal nucleation of microtubules (**9.19**). Vesicle-bound γ-tubulin was later also identified also in recycling Rab11-containing endosomes (82).

Association of GIT1 and βPIX with centrosomes was not unique for the mast cells, as it was observed in various cell types. Moreover, p21 protein (Cdc42/Rac)-activated kinase 1 (PAK1) was

also associated with interphase centrosomes in different cell types. While depletion of βPIX stimulated microtubule nucleation, depletion of GIT1 or PAK1 resulted in decreased nucleation. The importance of PAK1 for microtubule nucleation was corroborated by inhibition of its kinase activity. GIT1 with PAK1 thus represent positive regulators, and βPIX is a negative regulator of microtubule nucleation. Importantly, the regulatory roles of these signaling proteins in microtubule nucleation correlated with recruitment of γ-tubulin to the centrosome, while the general pericentriolar matrix integrity was unchanged. GIT1/βPIX signaling proteins were phosphorylated by PAK1 and directly interacted with γ-tubulin. The binding site for γ-tubulin on GIT1 was located into its N-terminal domain targeting GIT1 to the centrosome. Altogether, based on these data we suggested a novel regulatory mechanism of microtubule formation in interphase cells, in which GIT1 and βPIX signaling proteins, phosphorylated by the PAK1 kinase, modulate microtubule nucleation (**9.30**).

It has been well established that microtubules are implicated in actin-dependent cellular processes such as focal adhesion turnover, establishment of cell polarity, and migration (83). We found that profilin, controlling actin nucleation and assembly processes in eukaryotic cells, associates with microtubules via formins. Our study showed that profilin dynamically associates with microtubules, and this fraction of profilin contributes to balancing actin assembly during the cell growth and affects the microtubule dynamics. Depletion of profilin resulted in faster growth of microtubules. On the other hand, stimulation of actin dynamics reduced profilin association along the microtubules. The regulatory role of profilin in microtubule (+) end dynamics further support the conjecture of a close actin-microtubule interrelationship and underscoring profilin as a unique regulator of force generation and cellular behavior in eukaryotes (**9.31**). The profilin association with microtubules was independently confirmed later on (84).

4.3. Dysregulation of microtubule proteins in cancer cells

Tubulin is the target of some of the most widely used and time-honored anticancer TBAs. The clinical usefulness of many TBAs has been held back as a result of tumor cell drug resistance. The elucidation of the three-dimensional structure of αβ-tubulin dimer has provided an opportunity for rational drug design aimed at generating compounds that will target tubulin in therapeutically more efficacious ways compared to the presently available drugs. An issue to be addressed is which one(s) of the tubulin species, their isotypes, or their post-translationally modified forms should be specifically targeted in cancer chemotherapy. The βIII-tubulin is by far the most extensively studied isotype in human cancer. Early descriptive cellular/histopathological studies carried out during the 1990s have shown that the expression of βIII-tubulin is altered in cancer cells. At present, there is

general agreement that abnormal overexpression of βIII-tubulin in non-neuronal cancers is associated with an overall proclivity for aggressive tumor behavior and adverse clinical outcomes. Later on, studies of γ-tubulin expression revealed that this tubulin isotype is also dysregulated in some tumor types (**9.25**).

Gliomas are the most prevalent group of central nervous system neoplasms accounting for more than 70% of all brain tumors (85). Diffuse gliomas are particularly challenging forms of brain cancer owing to their highly invasive and infiltrative nature. Gliomas are broadly classified as low-grade (grades I and II) and high-grade gliomas (grades III and IV). High-grade gliomas are refractory to currently available treatments and carry a poor prognosis. Glioblastoma multiforme (GBM) is the most malignant, as well as the deadliest, glioma in adults. We found increased immunoreactivity for γ-tubulin in a panel of primary diffuse astrocytic gliomas (grades II-IV) and human glioblastoma cell lines. Immunoreactivity was significantly enhanced in high-grade anaplastic astrocytomas and GBM as compared to low-grade diffuse astrocytomas. In contrast to pericentrin, which localized to centrosomes, γ-tubulin was found both on centrosomes and in the cytosol. Our results indicated, for the first time, that overexpression and ectopic cellular distribution of γ-tubulin may be significant in the context of centrosome protein dysfunction and may be linked to tumor progression, where it may potentially serve as a novel marker of anaplastic change. This could also lay the foundation for a new approach to molecular stratification of gliomas (**9.15**).

Highly conserved β-tubulins of different classes differ in their C-terminal regions of molecules that are exposed on the outer surface of assembled microtubules. These C-terminal tails also harbor multiple PTMs. To specifically discriminate class III β-tubulin (βIII-tubulin), we generated a hybridoma producing anti-peptide mouse monoclonal antibody to peptide β441-448 from the C-terminal tail. Immunocytochemical staining revealed specific staining of cells of neuronal origin, and immunohistochemical examination of normal and neoplastic human tissues showed staining of cells from the central and peripheral nervous systems. Our findings thus provided evidence that βIII-tubulin is a useful marker for identification of cells of neuronal origin and neuronal neoplasms (**9.4**).

Although so-called "neuron-specific" βIII-tubulin is not expressed in non-transformed astrocytes and oligodendrocytes, its expression was not critically evaluated in the context of normal gliogenesis. We determined the expression of βIII-tubulin, in relation to other cytoskeletal marker proteins, in primary cultures of fetal astrocytes isolated from the cerebral hemispheres of human foetuses, and in autopsy brain sections derived from human fetuses of corresponding gestational ages. Our findings indicate that in conjunction with GFAP and nestin, βIII-tubulin is constitutively expressed in primary cultures of fetal astrocytes and progenitor-like cells of the

ventricular/subventricular zone and the cortical plate from the midgestational human brain. These data underscore the potential problems of phenotypic identity involving morphologically immature cells of the developing human brain, which exhibit immunoreactivity to βIII-tubulin (**9.18**).

Interestingly, βIII-tubulin was reported in many non-neuronal tumors including glioblastomas (86). Our immunohistochemical and immunofluorescence microscopic studies revealed that βIII-tubulin and γ-tubulin co-distributed in anaplastic astrocytomas and glioblastomas, and to a lesser extent, in low-grade diffuse astrocytomas. In glioblastoma cell lines, βIII-tubulin was associated with microtubules, whereas γ-tubulin exhibited striking diffuse cytoplasmic staining in addition to its expected centrosomal association. Treatment with different anti-microtubule drugs revealed that βIII-tubulin was not associated with insoluble γ-tubulin aggregates. On the other hand, immunoprecipitation experiments unveiled that both tubulins formed complexes in soluble cytoplasmic pools. We suggested that aberrant expression and interactions of βIII-tubulin and γ-tubulin might be linked to malignant changes in glial cells (**9.17**). These findings were confirmed by independent studies (87, 88).

Enhanced expression of γ-tubulin in cancer cells is not limited to gliomas. We found overexpression of γ-tubulin in medulloblastomas and in human medulloblastoma cell lines. In clinical tissue samples, the immunohistochemical distribution of γ-tubulin labeling was pervasive and inversely related to neuritogenesis. Overexpression of γ-tubulin was widespread in poorly differentiated, proliferating tumor cells, but was significantly diminished in quiescent differentiating tumor cells undergoing neuritogenesis, highlighted by βIII-tubulin immunolabeling. Overexpression of γ-tubulin in the context of medulloblastomas may be a molecular signature of phenotypic dedifferentiation (anaplasia) and may be linked to tumor progression and worse clinical outcomes (**9.20**).

Our original findings of elevated expression of γ-tubulins in some tumors were expanded in further studies, and increased expression and ectopic soluble distribution of γ-tubulin with decoupling/dissociation from the centrosomes was reported in non-small cell lung cancer (55), aggressive breast cancer cells (89, 90) and squamous cell carcinoma of the larynx (91). Furthermore, centrosome amplification closely linked to increased γ-tubulin cell content is present in many cancer cells (92). However, large clinical randomized studies are necessary to further elucidate the prognostic or predictive value of γ-tubulin in the context of different clinical stages, histological types, tumor grades, and treatment settings.

As some glioblastoma cell lines display high amounts of γ-tubulin, we performed a more detailed study of γ-tubulin subcellular distribution in these cells. γ-Tubulin was assumed to be a typical cytosolic protein necessary for nucleation of microtubules from microtubule organizing

centers. We obtained evidence that γ-tubulin is also present in the nucleoli of mammalian interphase cells. Immunoelectron microscopy revealed γ-tubulin localization outside fibrillar centers where transcription of ribosomal DNA takes place. γ-Tubulin was associated with nucleolar remnants after nuclear envelope breakdown and could be translocated to the nucleoli during mitosis. Pre-treatment of cells with leptomycin B did not affect the distribution of nuclear γ-tubulin, making it unlikely that rapid active transport via nuclear pores participates in the transport of γ-tubulin into the nucleus. Immunoprecipitation from nuclear extracts combined with mass spectrometry revealed an association of γ-tubulin with CDK5 regulatory subunit-associated tumor suppressor protein 3 (CDK5RAP3, C53). C53 located at multiple subcellular compartments including nucleoli. Overexpression of γ-tubulin antagonized the inhibitory effect of C53 on DNA damage G2/M checkpoint activation. The combined results indicated, for the first time, that aside from its known role in microtubule nucleation, γ-tubulin may also have nuclear-specific function(s) in animal cells (**9.23**). At the same time, nuclear γ-tubulin was identified in other cell types (45). Thereafter, it was suggested that nuclear γ-tubulin could play a role of transcription factor (93).

In addition to γ-tubulin, we also analyzed the expression, cellular distribution, and subcellular sorting of the γTuSC proteins GCP2 and GCP3 in glioblastoma cells. RT-qPCR and quantitative immunoblotting revealed a significant increase in the expression of GCP2 and GCP3 in glioblastoma cells versus normal human astrocytes. Both proteins were concentrated in the centrosomes in interphase cells, but punctate and diffuse localizations were also detected in the cytosol and nuclei/nucleoli. GCP2 and GCP3 formed complexes with γ-tubulin in the nucleoli as confirmed by reciprocal immunoprecipitation experiments and immunoelectron microscopy. GCP2 and GCP3 depletion caused accumulation of cells in G2/M and mitotic delay, but did not affect nucleolar integrity. Similarly, as in the case of γ-tubulin overexpression, the overexpression of GCP2 antagonized the inhibitory effect of C53 on the DNA damage G2/M checkpoint activity. Immunoreactivity for GCP2 and GCP3 was significantly increased over that in normal brains in glioblastoma tissue samples). These findings suggest that γTuSC protein dysregulation in glioblastomas may be linked to altered transcriptional checkpoint activity or interaction with signaling pathways associated with a malignant phenotype (**9.29**).

Given the role of microtubules on the invasive properties of tumor cells and the high propensity of GBM cells for brain invasion, we hypothesized that the microtubule-severing ATPase spastin might be aberrantly expressed or regulated in these cells. To test this hypothesis, we evaluated the expression levels and the intracellular distribution of spastin in human GBM cell lines and surgically excised tumor samples representative of all grades of diffuse astrocytic gliomas. In adult human brains, spastin was distributed predominantly in neurons and to a lesser extent, in glia.

Compared with normal mature brain tissues, the spastin expression and cellular distribution were increased in neoplastic glial phenotypes, especially in glioblastoma. Spastin was enriched in the leading edges of T98G glioblastoma cells. RT-qPCR and quantitative immunoblotting revealed higher levels of spastin in the glioblastoma cell lines versus normal human astrocytes. Functional experiments revealed that spastin depletion resulted in reduced cell motility and higher cell proliferation of T98G cells. Our results indicated, for the first time, that spastin expression in glioblastomas might be linked to tumor cell motility, migration, and invasion (**9.22**).

5. Conclusions

(Numbers in brackets refer to publications in Section 9)

1. We have prepared well-characterized monoclonal antibodies to γ-tubulin (2), βIII-tubulin (4) and GCP2 protein (29). The antibodies were extensively applied not only in studies of expression of these proteins during the development and regulation of microtubule nucleation, but also in immunohistochemical studies of different tumor types.

2. We have shown that βIII-tubulin is one of the first tissue-specific proteins in the course of neuronal differentiation (4, 14) and can be used for detection of tumors of neuronal origin (4). However, βIII-tubulin is also constitutively expressed, in conjunction with GFAP and nestin in primary astrocytes (18) and some non-neuronal tumors (17, 20). This suggests that the expression of βIII-tubulin cannot be considered as a unique proof of neuronal differentiation.

3. We have found that γ-tubulin is not a universal nucleator of microtubules and that other (-) end associated proteins can be involved in this process (2). We have proved association of γ-tubulin with the kinetochore/centromeric region in acentriolar plant cells (3, 5, 7) and its redistribution during erythrocyte differentiation (9) and cell division in protozoa (8, 12). In acentriolar cells, γ-tubulin could be involved in the modulation/stabilization of chromosome-microtubule interactions in the mitotic spindle.

4. Although two mammalian γ-tubulin isotypes are highly conserved, we were capable to discriminate them on 2D-PAGE in mouse (24) and human (33) cell lines and tissues. Both γ-tubulin-1 and γ-tubulin-2 are nucleation competent, but their expression substantially differs during mouse preimplantation development (24). Accumulation of γ-tubulin-2 in mature neurons and neuroblastoma during oxidative stress may signify its prosurvival function, whereas the main function of the dominant γ-tubulin-1 appears to be centered on microtubule nucleation (33). Investigation of human *TUBG1* missense mutations (resulting in malformations of brain cortical development) in the mouse model revealed that disease-related *TUBG1* variants exert their pathogenicity by affecting microtubule dynamics rather than centrosomal positioning or nucleation ability (37).

5. γ-Tubulin is capable to generate oligomers (10, 34), forms complexes with αβ-tubulin dimers (9, 10, 11, 17), and its binding properties change during the differentiation processes (9). These findings indicate that, apart from large (γTuRC) and small (γTuSC) complexes, other

molecular forms of γ-tubulin exist. γ-Tubulin is post-translationally modified (9, 10, 12, 14, 23, 24, 33), and these modifications might regulate interactions of γ-tubulin with αβ-tubulin dimers, GCPs, or the other proteins associated with γ-tubulin.

6. γ-Tubulin can form complexes with protein tyrosine kinases of Src (6, 14, 16, 19) or Syk (16, 36) families, protein tyrosine phosphatase SHP-1 (36) and phosphatidylinositol-3-kinase (PI3K) (19), which are involved in signal transduction. Moreover, proteins phosphorylated on tyrosine are included in γ-tubulin immunocomplexes of activated mast cells (6, 16). We proved phosphorylation of γ-tubulin and verified that selective inhibition of Src family kinases reduce the amount of phosphorylated γ-tubulin (14). Tyrosine kinases and phosphatases therefore could play important role in the regulation of γ-tubulin interactions.

7. We have found intrinsic association of γ-tubulin with membranes (11, 19, 34), including outer and inner mitochondrial membranes (33). Moreover, γ-tubulin is present in detergent-resistant membrane domains in cells undergoing neuronal differentiation and in mature neurons (19, 33). We have revealed that membrane-bound γ-tubulin complexes promote microtubule nucleation (11, 19). γ-Tubulin directly binds to the p85 regulatory domain of PI3K and microtubule nucleation from membranes is dependent on the activity of Src-family kinases and PI3K (19).

8. Activation of bone marrow-derived mast cells (BMMCs) results in rapid and transient reorganization of microtubules, generation of protrusion containing microtubules (21, 35, 36), and changes in microtubule (+) end dynamics (21). The formation of protrusions is dependent on the influx of extracellular Ca^{2+} and activity of STIM1 (21). The Ca^{2+} level also affects microtubule nucleation, γ-tubulin properties, and its interaction with GCPs (28). Inhibition of Ca^{2+}-dependent PKC kinases blocks generation of microtubule protrusions (35). Microtubule nucleation both in BMMCs and other cell types is regulated by GIT1/βPIX signaling proteins and PAK1 kinase (28, 30), which are generally considered to be microfilament regulators. We have found that profilin, controlling actin nucleation, dynamically associates with microtubules via formins. This fraction of profilin contributes to balancing actin assembly during the cell growth and affects the microtubule (+) end dynamics. Such regulatory role of profilin suggests a close actin-microtubule interrelationship (31).

9. We have found enhanced expression of γ-tubulin in gliomas (15, 17) and medulloblastomas (20). Moreover, increased levels of βIII-tubulin (17), GCP2/GCP3 (29) and microtubule-severing

ATPase spastin were characteristic features of highly motile glioblastoma cells, in which spastin depletion resulted in inhibition of cellular motility (22). Both γ-tubulin and GCP2/GCP3 were identified in the nucleus/nucleolus of glioblastoma cells, and overexpression of γ-tubulin or GCP2 antagonized the inhibitory effect of tumor suppressor CDK5RAP3 on the DNA damage G2/M checkpoint activity (23, 29). Dysregulation of microtubule nucleation proteins may be linked to altered transcriptional checkpoint activity or interaction with signaling pathways associated with a malignant phenotype.

10. We have developed methods for long-term storage of labile monoclonal antibodies (1) at ambient temperature and quantification of antibodies in hybridoma tissue culture media (13). We have developed highly sensitive and cost-effective assays for quantification of tau proteins (27).

6. Practical application of the results

(Numbers in brackets refer to publications in Section 9)

The results presented in this collection of publications increased our understanding of γ-tubulin subcellular distribution, its interacting proteins, and regulation of microtubule nucleation and organization in normal cells and under pathological conditions. The prepared monoclonal antibodies, constructs, stable cell lines, and developed methods have been used in many laboratories in the field.

In particular, well-characterized monoclonal antibodies contributed to advancement of cytoskeletal research in Czechoslovakia/Czech Republic. Many of prepared antibodies are used as world-wide standards and they are commercially available from various distributors (e.g., Abcam, Abnova, Biolegend, Cell Signalling, Exbio, GeneTex, Merck, Millipore, Novus Biologicals, Santa Cruz Biotechnology, Sigma-Aldrich, Thermo Fisher Scientific). Antibodies to neuron-specific microtubule proteins could be used for monitoring of neurodegenerative diseases, characterized by degradation of neurons (Alzheimer's and Parkinson's disease, multiple sclerosis), when fragments of microtubule proteins can be detected in body fluids. Monoclonal antibody TU-20 to βIII-tubulin easily recognizes epitopes on formaldehyde-fixed and paraffin-embedded material. It is therefore routinely used in clinical immunohistochemical detection of tumors of neuronal origin (4).

At present, there is a clear tendency to substitute production of monoclonal antibodies in laboratory animals by their preparation *in vitro*. The developed general method for simple, rapid and cost-effective quantification of antibodies at concentrations higher than 50 ng/ml in hybridoma tissue culture media has required sensitivity and can easily process a large number of samples (13). The method for stabilization of labile antibodies in the presence disaccharide trehalose allows

convenient long-term storage of large quantities of freeze-dried antibodies at ambient temperature, and thus facilitates their transport and simplifies the construction of pre-aliquoted kits based on such antibodies. The financial requirements for transport are substantially lower and the need for equipment to main low temperature is eliminated (1). The same method can be applied to the other labile proteins as we demonstrated in the case of tubulins.

The newly developed sensitive assay for quantitation of tau protein (important biomarker of Alzheimer's disease), based on sandwich ELISA with gold nanoparticles functionalized with tau-specific monoclonal antibody and oligonucleotide template (Nano-iPCR), allows detection of the tau protein at concentrations 5 pg/ml. This low-cost method is superior in sensitivity and detection range when compared to ELISA for tau protein detection (27).

Our results indicate that ectopic cellular expression of γ-tubulin in diffuse astrocytic gliomas is significantly increased in high-grade anaplastic astrocytomas and GBM (grade III-IV) as compared to low-grade diffuse astrocytomas (grade II) (15). As increased γ-tubulin amounts were observed in other tumor types, changes in γ-tubulin expression may be significant in the context of tumor progression, where it may potentially serve as a novel marker of anaplastic change.

Diseases associated with deregulated mast cell functions are hard to treat, and so the demand for new and better treatments targeting mast cell activation pathways increases. We have shown that miltefosine, a promising candidate for novel therapeutic strategies in mast cell-driven diseases, modulates mast cells both at the plasma membrane and in the cytosol. This alters intracellular signaling pathway(s) directed to microtubules, degranulation, and cell migration (35). Interference with the microtubular network via specific regulators of microtubule nucleation or dynamics could open up rational new approaches to the treatment of inflammatory and allergic diseases.

7. Future perspectives

The structural studies on γTuC have been highly illustrative, but a high-resolution structure of the γTuRC both before and after nucleation will be necessary to understand how GCP4-6 locate in the complex and what are the specific interactions they make with each other and with the γTuSC. It remains to be determined what factors are involved in promoting transition from the open to the closed state of γTuRCs. Elucidation of the precise mechanism of γTuRC activation also remains a pressing question in understanding its regulation. Many proteins interacting with γTuRCs have been implicated in the activation, targeting, and modulation of γTuRCs. However, little is known about the upstream signaling pathways ensuring that these proteins initiate microtubule nucleation

at the correct location and time. The importance of kinases and phosphatases in the regulation of nucleation is emerging, and therefore functional characterization of phosphorylation sites in γTuRC and targeting/anchoring proteins will be required. Another important issue that needs to be addressed is characterization of γTuRC subpopulations that might differ in composition or PTMs. Future studies are also necessary to find out whether distinct γTuRCs may be independently used by different tissues to generate cell-type-specific non-centrosomal microtubule arrays. The thorough understanding of microtubule nucleation should clarify the relevance of γTuRC dysregulation in cancer cells and neurodevelopmental diseases (34).

Currently, the most common and most effective chemotherapy compounds are TBAs that bind microtubules directly. Targeting γTuRCs may offer a viable alternative to perturbing cancer cells. γ-Tubulin has a binding site for colchicine, and the first γ-tubulin inhibitor Gatastatin, which displays higher affinity to γ-tubulin than to αβ-tubulin dimer, was developed by testing derivatives of TBAs (94). A future challenge will be to develop drugs that can inhibit the γTuRC function in a highly specific manner. In this respect, non-essential γTuRC proteins could provide good targets for anticancer drugs, as their inhibition may affect only a subset of γTuRCs.

In recent years, nucleation-independent functions of γ-tubulin have been getting more attention. High-resolution cryo-electron microscopy will be essential for deciphering the structure of recently reported γ-tubulin fibers and their high-order assemblies in the cellular context. The reconstitution of fibers *in vitro* and super-resolution microscopic techniques should help to understand the assembly principles for γ-tubulin fiber generation. It will also be important to mechanistically understand how γ-tubulin affects the (+) end dynamics, and the role of +TIPs and motor proteins in this process. Further studies are needed to assess whether self-repair of microtubules by insertion of GTP tubulin dimers into the microtubule lattice is linked with the potential γ-tubulin role as rescue factor (95). Understanding the role of γ-tubulins under various stress conditions, in DNA damage checkpoints and signaling pathways to DNA repair will be important to elucidate the role of γ-tubulin in tumorigenesis.

It is becoming increasingly clear that microtubules and actin filaments often work together in core cellular processes and their dynamic properties are often intertwined. Recent works implicate that both microtubules and actin are nucleated from centrosomes and actin tunes the microtubule nucleation (96). Sophisticated *in vitro* reconstitution assays should reveal what is the role of microtubule nucleating and regulatory proteins in the cross-talks between these two cytoskeletal systems.

Finally, mast cells represent a useful model system for studying rapid and transient reorganization of microtubules in response to antigen-stimulated activation. It will be crucial to

understand the Ca^{2+}-dependent signaling pathway(s) that modulate microtubule nucleation, and microtubule (+) end dynamics. High-speed super-resolution live-cell imaging should help to illuminate these events, which are relevant for mast cell degranulation. This could lead to development of new approaches to the treatment of mast cell-driven diseases.

8. References

1. Verhey, K. J., and J. Gaertig. 2007. The tubulin code. *Cell Cycle* 6: 2152-2160.
2. Nogales, E., S. G. Wolf, and K. H. Downing. 1998. Structure of the αβ tubulin dimer by electron crystallography. *Nature* 391: 199-203.
3. Nogales, E., and H. W. Wang. 2006. Structural mechanisms underlying nucleotide-dependent self-assembly of tubulin and its relatives. *Curr. Opin. Struct. Biol.* 16: 221-229.
4. Löwe, J., H. Li, K. H. Downing, and E. Nogales. 2001. Refined structure of αβ-tubulin at 3.5 Å resolution. *J. Mol. Biol.* 313: 1045-1057.
5. Li, H., D. J. DeRosier, W. V. Nicholson, E. Nogales, and K. H. Downing. 2002. Microtubule structure at 8 Å resolution. *Structure* 10: 1317-1328.
6. Wade, R. H., and A. A. Hyman. 1997. Microtubule structure and dynamics. *Curr. Opin. Cell Biol.* 9: 12-17.
7. Alushin, G. M., G. C. Lander, E. H. Kellogg, R. Zhang, D. Baker, and E. Nogales. 2014. High-resolution microtubule structures reveal the structural transitions in αβ-tubulin upon GTP hydrolysis. *Cell* 157: 1117-1129.
8. Downing, K. H. 2000. Structural basis for the interaction of tubulin with proteins and drugs that affect microtubule dynamics. *Annu. Rev. Cell Dev. Biol.* 16: 89-111.
9. Field, J. J., A. Kanakkanthara, and J. H. Miller. 2014. Microtubule-targeting agents are clinically successful due to both mitotic and interphase impairment of microtubule function. *Bioorg. Med. Chem.* 22: 5050-5059.
10. Mitchison, T., and M. Kirschner. 1984. Dynamic instability of microtubule growth. *Nature* 312: 237-242.
11. Akhmanova, A., and M. O. Steinmetz. 2015. Control of microtubule organization and dynamics: two ends in the limelight. *Nat. Rev. Mol. Cell Biol.* 16: 711-726.
12. Coombes, C. E., A. Yamamoto, M. R. Kenzie, D. J. Odde, and M. K. Gardner. 2013. Evolving tip structures can explain age-dependent microtubule catastrophe. *Curr. Biol.* 23: 1342-1348.
13. Dimitrov, A., M. Quesnoit, S. Moutel, I. Cantaloube, C. Poüs, and F. Perez. 2008. Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. *Science* 322: 1353-1356.
14. Aumeier, C., L. Schaedel, J. Gaillard, K. John, L. Blanchoin, and M. Théry. 2016. Self-repair promotes microtubule rescue. *Nat. Cell Biol.* 18: 1054-1064.
15. Ludueña, R. F. 1998. Multiple forms of tubulin: different gene products and covalent modifications. *Int. Rev. Cytol.* 178: 207-275.
16. Downing, K. H., and E. Nogales. 1998. Tubulin and microtubule structure. *Curr. Opin. Cell Biol.* 10: 16-22.
17. Roll-Mecak, A. 2019. How cells exploit tubulin diversity to build functional cellular microtubule mosaics. *Curr. Opin. Cell Biol.* 56: 102-108.
18. Dutcher, S. K. 2003. Long-last relatives reappear: identification of new members of the tubulin superfamily. *Curr. Opin. Microbiol.* 6: 634-640.

19. Ludueña, R. F., and A. Banerjee. 2008. The tubulin superfamily. In *The role of microtubules in cell biology, neurobiology and oncology*. T. Fojo, ed. Humana Press, Totowa, NJ. 177-191.
20. Libusová, L., and P. Dráber. 2006. Multiple tubulin forms in ciliated protozoan *Tetrahymena* and *Paramecium* species. *Protoplasma* 227: 65-76.
21. Jordan, M. A., and K. Kamath. 2007. How do microtubule-targeted drugs work? An overview. *Curr. Cancer Drug Targets* 7: 730-742.
22. Kavallaris, M. 2010. Microtubules and resistance to tubulin-binding agents. *Nat. Rev Cancer* 10: 194-204.
23. Ludueña, R. F., and A. Banerjee. 2008. The isotypes of tubulin: distribution and functional significance. In *The role of microtubules in cell biology, neurobiology and oncology*. T. Fojo, ed. Humana Press, Totowa, NJ. 123-175.
24. Joe, P. A., A. Banerjee, and R. F. Ludueña. 2009. Roles of β-tubulin residues Ala^{428} and Thr^{429} in microtubule formation *in vivo*. *J. Biol. Chem.* 284: 4283-4291.
25. Katsetos, C. D., E. Dráberová, A. Legido, C. Dumontet, and P. Dráber. 2009. Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. I. Class III β-tubulin. *J. Cell Physiol.* 221: 505-513.
26. Gadadhar, S., S. Bodakuntla, K. Natarajan, and C. Janke. 2017. The tubulin code at a glance. *J. Cell Sci.* 130: 1347-1353.
27. Dráber, P., and E. Dráberová. 2012. Microtubules. In *Cytoskeleton and Human Disease*. M. Kavallaris, ed. Humana Press, New York: NY. 29-54.
28. Maurer, S. P., N. I. Cade, G. Bohner, N. Gustafsson, E. Boutant, and T. Surrey. 2014. EB1 accelerates two conformational transitions important for microtubule maturation and dynamics. *Curr. Biol.* 24: 372-384.
29. Hendershott, M. C., and R. D. Vale. 2014. Regulation of microtubule minus-end dynamics by CAMSAPs and Patronin. *Proc. Natl. Acad. Sci. USA* 111: 5860-5865.
30. Jiang, K., S. Hua, R. Mohan, I. Grigoriev, K. W. Yau, Q. Liu, E. A. Katrukha, A. F. Altelaar, A. J. Heck, C. C. Hoogenraad, and A. Akhmanova. 2014. Microtubule minus-end stabilization by polymerization-driven CAMSAP deposition. *Dev. Cell* 28: 295-309.
31. Oakley, C. E., and B. R. Oakley. 1989. Identification of γ-tubulin, a new member of the tubulin superfamily encoded by mipA gene of *Aspergillus nidulans*. *Nature* 338: 662-664.
32. Wise, D. O., R. Krahe, and B. R. Oakley. 2000. The γ-tubulin gene family in humans. *Genomics* 67: 164-170.
33. Yuba-Kubo, A., A. Kubo, M. Hata, and S. Tsukita. 2005. Gene knockout analysis of two γ-tubulin isoforms in mice. *Dev. Biol.* 282: 361-373.
34. Sulimenko, V., Z. Hájková, A. Klebanovych, and P. Dráber. 2017. Regulation of microtubule nucleation mediated by γ-tubulin complexes. *Protoplasma* 254: 1187-1199.
35. Murphy, S. M., L. Urbani, and T. Stearns. 1998. The mammalian γ-tubulin complex contains homologues of the yeast spindle pole body components Spc97p and Spc98p. *J. Cell Biol.* 141: 663-674.
36. Kollman, J. M., J. K. Polka, A. Zelter, T. N. Davis, and D. A. Agard. 2010. Microtubule nucleating γ-TuSC assembles structures with 13-fold microtubule-like symmetry. *Nature* 466: 879-882.
37. Teixidó-Travesa, N., J. Villén, C. Lacasa, M. T. Bertran, M. Archinti, S. P. Gygi, C. Caelles, J. Roig, and Lüders. 2010. The γTuRC revisited: a comparative analysis of interphase and mitotic human γTuRC redefines the set of core components and identifies the novel subunit GCP8. *Mol. Biol. Cell* 21: 3963-3972.
38. Kollman, J. M., A. Merdes, L. Mourey, and D. A. Agard. 2011. Microtubule nucleation by γ-tubulin complexes. *Nat. Rev. Mol. Cell Biol.* 12: 709-721.

39. Wieczorek, M., S. Bechstedt, S. Chaaban, and G. J. Brouhard. 2015. Microtubule-associated proteins control the kinetics of microtubule nucleation. *Nat. Cell Biol.* 17: 907-916.
40. Lawo, S., M. Hasegan, G. D. Gupta, and L. Pelletier. 2012. Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material. *Nat. Cell Biol.* 14: 1148-1158.
41. Petry, S., and R. D. Vale. 2015. Microtubule nucleation at the centrosome and beyond. *Nat. Cell Biol.* 17: 1089-1093.
42. Kollman, J. M., C. H. Greenberg, S. Li, M. Moritz, A. Zelter, K. K. Fong, J. J. Fernandez, A. Sali, J. Kilmartin, T. N. Davis, and D. A. Agard. 2015. Ring closure activates yeast γTuRC for species-specific microtubule nucleation. *Nat. Struct. Mol. Biol.* 22: 132-137.
43. Choi, Y. K., P. Liu, S. K. Sze, C. Dai, and R. Z. Qi. 2010. CDK5RAP2 stimulates microtubule nucleation by the γ-tubulin ring complex. *J. Cell Biol.* 191: 1089-1095.
44. Oakley, B. R., V. Paolillo, and Y. Zheng. 2015. γ-Tubulin complexes in microtubule nucleation and beyond. *Mol. Biol. Cell* 26: 2957-2962.
45. Höög, G., R. Zarrizi, K. von Stedingk, K. Jonsson, and M. Alvarado-Kristensson. 2011. Nuclear localization of γ-tubulin affects E2F transcriptional activity and S-phase progression. *FASEB J.* 25: 3815-3827.
46. Ehlén, Å., C. A. Rosselló, K. von Stedingk, G. Höög, E. Nilsson, H. M. Pettersson, K. Jirström, and M. Alvarado-Kristensson. 2012. Tumors with nonfunctional retinoblastoma protein are killed by reduced γ-tubulin levels. *J. Biol. Chem.* 287: 17241-17247.
47. Chumová, J., L. Trögelová, H. Kourová, J. Volc, V. Sulimenko, P. Halada, O. Kučera, O. Benada, A. Kuchařová, A. Klebanovych, P. Dráber, G. Daniel, and P. Binarová. 2018. γ-Tubulin has a conserved intrinsic property of self-polymerization into double stranded filaments and fibrillar networks. *BBA Mol. Cell Res.* 1865: 734-748.
48. Chumová, J., H. Kourová, L. Trögelová, P. Halada, and P. Binarová. 2019. Microtubular and nuclear functions of γ-tubulin: are they LINCed? *Cells* 8.
49. Rosselló, C. A., L. Lindström, G. Eklund, M. Corvaisier, and M. A. Alvarado-Kristensson. 2018. γ-Tubulin-γ-tubulin interactions as the basis for the formation of a meshwork. *Int. J. Mol. Sci.* 19.
50. Dráberová, E., V. Sulimenko, S. Vinopal, T. Sulimenko, V. Sládková, L. D'Agostino, M. Sobol, P. Hozák, L. Křen, C. D. Katsetos, and P. Dráber. 2017. Differential expression of human γ-tubulin isotypes during neuronal development and oxidative stress points to a γ-tubulin-2 prosurvival function. *FASEB J.* 31: 1828-1846.
51. Lesca, C., M. Germanier, B. Raynaud-Messina, C. Pichereaux, C. Etievant, S. Emond, O. Burlet-Schiltz, B. Monsarrat, M. Wright, and M. Defais. 2005. DNA damage induce γ-tubulin-RAD51 nuclear complexes in mammalian cells. *Oncogene* 24: 5165-5172.
52. Zhang, S., P. Hernmerich, and F. Grosse. 2007. Centrosomal localization of DNA damage checkpoint proteins. *J. Cell. Biochem.* 101: 451-465.
53. Hubert, T., J. Vandekerckhove, and J. Gettemans. 2011. Cdk1 and BRCA1 target γ-tubulin to microtubule domains. *Biochem. Biophys. Res. Commun.* 414: 240-245.
54. Hořejší, B., S. Vinopal, V. Sládková, E. Dráberová, V. Sulimenko, T. Sulimenko, V. Vosecká, A. Philimonenko, P. Hozák, C. D. Katsetos, and P. Dráber. 2012. Nuclear γ-tubulin associates with nucleoli and interacts with tumor suppressor protein C53. *J. Cell Physiol.* 227: 367-382.
55. Katsetos, C. D., and P. Dráber. 2012. Tubulins as therapeutic targets in cancer: from bench to bedside. *Curr. Pharm. Design* 18: 2778-2792.
56. Remy, M. H., A. Merdes, and L. Gregory-Pauron. 2013. Assembly of gamma-tubulin ring complexes: implications for cell biology and disease. *Prog. Mol. Biol. Transl. Sci.* 117: 511-530.

57. Poirier, K., N. Lebrun, L. Broix, G. Tian, Y. Saillour, C. Boscheron, E. Parrini, S. Valence, B. S. Pierre, M. Oger, D. Lacombe, D. Geneviève, E. Fontana, F. Darra, C. Cances, M. Barth, D. Bonneau, B. D. Bernadina, S. N'Guyen, C. Gitiaux, P. Parent, V. des Portes, J. M. Pedespan, V. Legrez, L. Castelnau-Ptakine, P. Nitschke, T. Hieu, C. Masson, D. Zelenika, A. Andrieux, F. Francis, R. Guerrini, N. J. Cowan, N. Bahi-Buisson, and J. Chelly. 2013. Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat. Genet.* 45: 639-647.
58. Brock, S., K. Stouffs, E. Scalais, M. D'Hooghe, K. Keymolen, R. Guerrini, W. B. Dobyns, N. Di Donato, and A. C. Jansen. 2018. Tubulinopathies continued: refining the phenotypic spectrum associated with variants in TUBG1. *Eur. J. Hum. Genet.* 26: 1132-1142.
59. Ivanova, E. L., J. G. Gilet, V. Sulimenko, A. Duchon, G. Rudolf, K. Runge, S. C. Collins, L. Asselin, L. Broix, N. Drouot, P. Tilly, P. Nusbaum, A. Vincent, W. Magnant, V. Skory, M. C. Birling, G. Pavlovic, J. D. Godin, B. Yalcin, Y. Herault, P. Dráber, J. Chelly, and M. V. Hinckelmann. 2019. TUBG1 missense variants underlying cortical malformations disrupt neuronal locomotion and microtubule dynamics but not neurogenesis. *Nat. Commun.* 10: 2129.
60. Viklický, V., P. Dráber, J. Hašek, and J. Bártek. 1982. Production and characterization of a monoclonal antitubulin antibody. *Cell Biol. Int. Rep.* 6: 725-731.
61. Goding, J. W. 1996. *Monoclonal antibodies: principles and practice*. Academic Press, London.
62. Cordone, L., G. Cottone, A. Cupane, A. Emanuele, S. Giuffrida, and M. Levantino. 2015. Proteins in saccharides matrices and the trehalose pecularity: biochemical and biopysical properties. *Curr. Org. Chem.* 19: 1684-1706.
63. Shelanski, M. L., F. Gaskin, and C. R. Cantor. 1973. Microtubule assembly in the absence of added nucleotides. *Proc. Natl. Acad. Sci. USA* 70: 765-768.
64. Weingarten, M. D., A. H. Lockwood, S. Y. Hwo, and M. W. Kirschner. 1975. A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. USA* 72: 1858-1868.
65. Waterman-Storer, C. M. 2001. Microtubule/organelle motility assays. *Curr. Protoc. Cell Biol.*: 13.11.11-13.11.21.
66. Soifer, D., A. H. Laszlo, and J. M. Scotto. 1972. Enzymatic activity in tubulin preparations. I. Intrinsic protein kinase activity in lyophilized preparations of tubulin from porcine brain. *Biochim. Biophys. Acta* 271: 182-192.
67. Banerjee, A., M. C. Roach, P. Trcka, and R. F. Ludueña. 1990. Increased microtubule assembly in bovine brain tubulin lacking the type III isotype of β-tubulin. *J. Biol. Chem.* 265: 1794-1799.
68. Colello, D., C. G. Reverte, R. Ward, C. W. Jones, V. Magidson, A. Khodjakov, and S. E. LaFlamme. 2010. Androgen and Src signaling regulate centrosome activity. *J. Cell Sci.* 123: 2094-2102.
69. Joshi, H. C. 1993. γ-Tubulin: the hub of cellular microtubule assemblies. *BioEssays* 15: 637-643.
70. De Brabander, M., G. Geuens, R. Nuydens, R. Willebrords, F. Aerts, and J. De Mey. 1986. Microtubule dynamics during the cell cycle: the effects of taxol and nocodazole on the microtubule system of PtK2 cells at different stages of the mitotic cycle. *Int. Rev. Cytol.* 101: 215-274.
71. Lindström, L., T. Li, D. Malycheva, A. Kancharla, H. Nilsson, N. Vishnu, H. Mulder, M. Johansson, C. A. Rosselló, and M. Alvarado-Kristensson. 2018. The GTPase domain of gamma-tubulin is required for normal mitochondrial function and spatial organization. *Commun. Biol.* 1: e37.
72. Guerrini, R., and W. B. Dobyns. 2014. Malformations of cortical development: clinical features and genetic causes. *Lancet Neurol.* 13: 710-726.
73. Cavalier-Smith, T. 1998. A revised six-kingdom system of life. *Biol. Rev.* 73: 203-266.

74. Joshi, H. C., and B. A. Palevitz. 1996. γ-Tubulin and microtubule organization in plants. *Trends Cell Biol.* 6: 41-44.
75. Meunier, S., and I. Vernos. 2016. Acentrosomal microtubule assembly in mitosis: the where, when, and how. *Trends Cell Biol.* 26: 80-87.
76. Lindström, L., and M. Alvarado-Kristensson. 2018. Characterization of gamma-tubulin filaments in mammalian cells. *BBA Mol. Cell Res.* 1865: 158-171.
77. Cruse, G., M. A. Beaven, I. Ashmole, P. Bradding, A. M. Gilfillan, and D. D. Metcalfe. 2013. A truncated splice-variant of the FcεRIβ receptor subunit is critical for microtubule formation and degranulation in mast cells. *Immunity* 38: 906-917.
78. Munoz, I., L. Danelli, J. Claver, N. Goudin, M. Kurowska, I. K. Madera-Salcedo, J. D. Huang, A. Fischer, C. Gonzalez-Espinosa, B. G. de Saint, U. Blank, and G. Menasche. 2016. Kinesin-1 controls mast cell degranulation and anaphylaxis through PI3K-dependent recruitment to the granular Slp3/Rab27b complex. *J. Cell Biol.* 215: 203-216.
79. Gilfillan, A. M., and J. Rivera. 2009. The tyrosine kinase network regulating mast cell activation. *Immunol. Rev.* 228: 149-169.
80. Maurer, M., M. Magerl, M. Metz, K. Weller, and F. Siebenhaar. 2013. Miltefosine: a novel treatment option for mast cell-mediated diseases. *J. Dermatolog. Treat.* 24: 244-249.
81. Vogel, J., B. Drapkin, J. Oomen, D. Beach, K. Bloom, and M. Snyder. 2001. Phosphorylation of γ-tubulin regulates microtubule organization in budding yeast. *Dev. Cell* 1: 621-631.
82. Hehnly, H., and S. Doxsey. 2014. Rab11 endosomes contribute to mitotic spindle organization and orientation. *Dev. Cell* 28: 497-507.
83. Dogterom, M., and G. H. Koenderink. 2019. Actin-microtubule crosstalk in cell biology. *Nat. Rev. Mol. Cell Biol.* 20: 38-54.
84. Henty-Ridilla, J. L., M. A. Juanes, and B. L. Goode. 2017. Profilin directly promotes microtubule growth through residues mutated in amyotrophic lateral sclerosis. *Curr. Biol.* 27: 3535-3543.
85. Ohgaki, H., and P. Kleihues. 2005. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *J. Neuropathol. Exp. Neurol.* 64: 479-489.
86. Katsetos, C. D., A. Legido, E. Perentes, and S. J. Mörk. 2003. Class III β-tubulin isotype: a key cytoskeletal protein at the crossroads of developmental neurobiology and tumor neuropathology. *J. Child Neurol* 18: 851-866.
87. Loh, J. K., A. S. Lieu, C. H. Chou, C. C. Lin, M. C. Yang, F. Y. Lin, Y. R. Hong, and S. L. Howng. 2012. Differential expression of centrosome-associated proteins in human brain tumors: a possible role of hNinein isoform 6 in cell differentiation. *Biofactors* 38: 470-477.
88. Tsai, H. P., C. Y. Tsai, A. S. Lieu, C. Y. Chai, A. L. Kwan, S. L. Howng, and J. K. Loh. 2014. Association of Aurora A and γ-tubulin expression in astrocytomas and patient survival. *Neurol. Res.* 36: 746-751.
89. Niu, Y., T. Liu, G. M. Tse, B. Sun, R. Niu, H. M. Li, H. Wang, Y. Yang, X. Ye, Y. Wang, Q. Yu, and F. Zhang. 2009. Increased expression of centrosomal α, γ-tubulin in atypical ductal hyperplasia and carcinoma of the breast. *Cancer Sci.* 100: 580-587.
90. Cho, E. H., R. A. Whipple, M. A. Matrone, E. M. Balzer, and S. S. Martin. 2010. Delocalization of γ-tubulin due to increased solubility in human breast cancer cell lines. *Cancer Biol. Ther.* 9: 66-76.
91. Syed, M. I., S. Syed, F. Minty, S. Harrower, J. Singh, A. Chin, D. R. McLellan, E. K. Parkinson, and L. J. Clark. 2009. Gamma tubulin: a promising indicator of recurrence in squamous cell carcinoma of the larynx. *Otolaryngol. Head Neck Surg.* 140: 498-504.

92. Godinho, S. A., R. Picone, M. Burute, R. Dagher, Y. Su, C. T. Leung, K. Polyak, J. S. Brugge, M. Théry, and D. Pellman. 2014. Oncogene-like induction of cellular invasion from centrosome amplification. *Nature* 510: 167-171.
93. Lindström, L., B. O. Villoutreix, S. Lehn, R. Hellsten, E. Nilsson, E. Crneta, R. Olsson, and M. Alvarado-Kristensson. 2015. Therapeutic targeting of nuclear γ-tubulin in RB1-negative tumors. *Mol. Cancer Res.* 13: 1073-1082.
94. Chinen, T., P. Liu, S. Shioda, J. Pagel, B. Cerikan, T. C. Lin, O. Gruss, Y. Hayashi, H. Takeno, T. Shima, Y. Okada, I. Hayakawa, Y. Hayashi, H. Kigoshi, T. Usui, and E. Schiebel. 2015. The γ-tubulin-specific inhibitor gatastatin reveals temporal requirements of microtubule nucleation during the cell cycle. *Nat. Commun.* 6: 8722.
95. Bouissou, A., C. Verollet, H. de Forges, L. Haren, Y. Bellaiche, F. Perez, A. Merdes, and B. Raynaud-Messina. 2014. γ-Tubulin Ring Complexes and EB1 play antagonistic roles in microtubule dynamics and spindle positioning. *EMBO J.* 33: 114-128.
96. Farina, F., N. Ramkumar, L. Brown, D. Samandar Eweis, J. Anstatt, T. Waring, J. Bithell, G. Scita, M. Théry, L. Blanchoin, T. Zech, and B. Baum. 2019. Local actin nucleation tunes centrosomal microtubule nucleation during passage through mitosis. *EMBO J.* 38.

9. List of publications that form the basis of the dissertation

(IF of the year in which publication was published; citation from Web of Science without self-citations, 21.7.2019)

9.1. Dráber P., Dráberová E., Nováková M.: Stability of monoclonal IgM antibodies freeze-dried in the presence of trehalose. **J. Immunol. Meth.** 181: 37-43, 1995. (IF=1.901; 40)

9.2. Nováková M., Dráberová E., Schürmann W., Czihak G., Viklický V., Dráber P.: γ-Tubulin redistribution in taxol-treated mitotic cells probed by monoclonal antibodies. **Cell Motil. Cytoskel.** 33: 38-51, 1996. (IF=2.637; 25)

9.3. Binarová P., Hause B., Doležel J., Dráber P.: Association of γ-tubulin with kinetochore/centromeric region of plant chromosomes. **Plant J.** 14: 751-757, 1998. (IF=5.765; 39)

9.4. Dráberová E., Lukáš Z., Ivanyi D., Viklický V., Dráber P.: Expression of class III β-tubulin in normal and neoplastic human tissues. **Histochem. Cell Biol.** 109: 231-239, 1998. (IF=1.687; 74)

9.5. Binarová P., Doležel J., Dráber P., Heberle-Bors E., Strnad M., Bögre L: Treatment of *Vicia fa*ba root tip cells with specific inhibitors to cyclin-dependent kinases leads to abnormal spindle formation. **Plant J.** 16: 697-702, 1998. (IF=5.765; 53)

9.6. Dráberová L., Dráberová E., Surviladze Z., Dráber Pe., Dráber P.: Protein tyrosine kinase p53/p56^{lyn} forms complexes with γ-tubulin in rat basophilic leukemia cells. **Int. Immunol.** 11: 1829-1839, 1999. (IF=2.897; 8)

9.7. Binarová P., Cenklová V., House B., Kubátová E., Lysák M., Doležel J., Bögre L., Dráber P.: Nuclear γ-tubulin during acentriolar plant mitosis. **Plant Cell** 12: 433-442, 2000. (IF=11.093; 43)

9.8. Nohýnková E., Dráber P., Reishig J., Kulda J.: Localization of gamma-tubulin in interphase and mitotic cells of a unicellular eukaryote, *Giardia intestinalis*. **Eur. J. Cell Biol.** 79: 438-445, 2000. (IF=2.801; 43)

9.9. Linhartová I., Novotná B., Sulimenko V., Dráberová E., Dráber P.: Gamma-tubulin in chicken erythrocytes: changes in localization during cell differentiation and characterization of cytoplasmic complexes. **Develop. Dyn.** 223: 229-240, 2002. (IF=3.804; 5)

9.10. Sulimenko V., Sulimenko T., Poznanovic S., Nechiporuk-Zloy V., Böhm K., Macůrek L., Unger E., Dráber P.: Association of brain γ-tubulins with αβ-tubulin dimers. **Biochem. J.** 365: 889-895, 2002. (IF=4.589; 8)

9.11. Dryková D., Sulimenko V., Cenklová V., Volc J., Dráber P., Binarová P.: Plant γ-tubulin interacts with αβ-tubulin dimers and forms membrane-associated complexes. **Plant Cell** 15: 465-480, 2003. (IF=10.679; 74)

9.12. Libusová L., Sulimenko T., Sulimenko V., Hozák P., Dráber P. γ-Tubulin in *Leishmania*: cell-cycle dependent changes in subcellular localization and heterogeneity of its isoforms. **Exp. Cell Res.** 295: 375-386, 2004. (IF=4.007; 15)

9.13. Sulimenko T., Dráber P.: A fast and simple dot-immunobinding assay for quantification of mouse immunoglobulins in hybridoma culture supernatants. **J. Immunol. Meth.** 289: 89-95, 2004. (IF=2.464; 14)

9.14. Kukharskyy V., Sulimenko V., Macůrek L., Sulimenko T., Dráberová E., Dráber P.: Complexes of γ-tubulin with nonreceptor protein tyrosin kinases Src and Fyn in differentiating P19 embryonal carcinoma cells. **Exp. Cell Res.** 298: 218-228, 2004. (IF=4.007; 23)

9.15. Katsetos C. D., Reddy G., Dráberová E., Šmejkalová B., Del Valle L., Ashraf Q., Tadevosyan A., Yelin K., Maraziotis T., Mishra O. P., Mörk S., Legido A., Nissanov J., Baas P. W., de Chadarévian J. P., Dráber P.: Altered cellular distribution and subcellular sorting of γ-tubulin in diffuse astrocytic gliomas and human glioblastoma cell lines. **J. Neuropathol. Exp. Neurol.** 65: 465-477, 2006. (IF=4.371; 16)

9.16. Sulimenko V., Dráberová E., Sulimenko T., Macůrek L., Richterová V., Dráber Pe., Dráber P.: Regulation of microtubule formation in activated mast cells by complexes of γ-tubulin with Fyn and Syk kinases. **J. Immunol.** 176: 7243-7253, 2006. (IF=6.293; 33)

9.17. Katsetos C. D., Dráberová E., Šmejkalová B., Reddy G., Bertrand L., de Chadarévian J. P., Legido A., Nissanov J., Baas P.W., Dráber P.: Class III β-tubulin and γ-tubulin are co-expressed and form complexes in human glioblastoma cells. **Neurochem. Res.** 32: 1387-1398, 2007. (IF=1.811; 34)

9.18. Dráberová E., Del Valle L., Gordon J., Marková V., Šmejkalová B., Bertrand L., de Chadarévian J. P., Agamanolis, D. P., Legido A., Khalili K., Dráber P., Katsetos C.D.: Class III β-tubulin is constitutively co-expressed with GFAP and nestin in midgestational human fetal astrocytes: implications in phenotypic identity. **J. Neuropathol. Exp. Neurol.** 67: 341-354, 2008. (IF=5.140; 62)

9.19. Macůrek L., Dráberová E., Richterová V., Sulimenko V., Sulimenko T., Dráberová L., Marková V., Dráber P.: Regulation of microtubule nucleation in differentiating embryonal carcinoma cells by complexes of membrane-bound γ-tubulin with Fyn kinase and phosphoinositide 3-kinase. **Biochem. J.** 416: 421-430, 2008. (IF=4.371; 23)

9.20. Caracciolo V., D'Agostino L., Dráberová E., Sládková V., Crozier-Fitzgerald C., Agamanolis D. P., de Chadarévian J. P., Legido A., Giordano A., Dráber P., Katsetos C. D.: Differential expression and cellular distribution of γ-tubulin and βIII-tubulin in medulloblastomas and human medulloblastoma cell lines. **J. Cell Physiol.** 223: 519-529, 2010. (IF=3.986; 21)

9.21. 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.** 186: 913-923, 2011. (IF=5.788; 17)

9.22. Dráberová E., Vinopal S., Morfini G., Liu P.S., Sulimenko T., Sládková V., Burns M.R., Solowska J., Kulandaivel K., de Chadarévian J.-P., Legido A., Mörk S.J., Janáček J.,

Baas P.W., Dráber P., Katsetos C. D.: Microtubule-severing ATPase spastin in glioblastoma: increased expression in human glioblastoma cell lines and inverse roles in cell motility and proliferation**. J. Neuropathol. Exp. Neurol.** 70: 811-826, 2011. (IF=4.258; 14)

9.23. Hořejší B., Vinopal S., Sládková V., Dráberová E., Sulimenko V., Sulimenko T., Vosecká V., Philimonenko A., Hozák P., Katsetos C. D., Dráber P.: Nuclear γ-tubulin associates with nucleoli and interacts with tumor suppressor protein C53. **J. Cell Physiol.** 227: 367-382, 2012. (IF=4.218; 19)

9.24. Vinopal S., Černohorská M, Sulimenko V., Sulimenko T., Vosecká V., Flemr M., Dráberová E., Dráber P.: γ-Tubulin 2 nucleates microtubules and is downregulated in mouse early embryogenesis. **PLoS ONE** 7: e29919, 2012. (IF=3.730; 9)

9.25. Katsetos C.D., Dráber P.: Tubulins as therapeutic targets in cancer: from bench to bedside. **Curr. Pharm. Design** 18: 2778-2792, 2012. (IF=3.311; 43)

9.26. Dráber P., Sulimenko V., Dráberová E.: Cytoskeleton in mast cell signaling. **Front. Immunol.** 3: e130, 2012. (IF=5.695; 21)

9.27. Stegurová L., Dráberová E., Bartoš A., Dráber P., Řípová D., Dráber Pe.: Gold-nanoparticle-based immuno-PCR for detection of tau protein in cerebrospinal fluid. **J. Immunol. Meth.** 406: 137-142, 2014. (IF=1.820; 15)

9.28. Sulimenko V., Hájková Z., Černohorská M., Sulimenko T., Sládková V., Dráberová L., Vinopal S., Dráberová E., Dráber P.: Microtubule nucleation in mouse bone marrow-derived mast cells is regulated by concerted action of GIT1/βPIX proteins and calcium. **J. Immunol.** 194: 4099-4111, 2015. (IF=4.985; 1)

9.29. Dráberová E., D'Agostino, L., Caracciolo V., Sládková V., Sulimenko T., Sulimenko V., Sobol M., Maounis N.F., Tzelepis E.G., Mahera E., Křen L, Legido A., Giordano A., Mörk S., Hozák P., Dráber P. Katsetos C.D: Overexpression and nucleolar localization of γ-tubulin small complex proteins GCP2 and GCP3 in glioblastoma. **J. Neuropathol. Exp. Neurol.** 74: 723-742, 2015. (IF=3.432; 9)

9.30. Černohorská M., Sulimenko V., Hájková Z., Sulimenko T., Sládková V., Vinopal S., Dráberová E., Dráber P.: GIT1/βPIX signaling proteins and PAK1 kinase regulate microtubule nucleation. **BBA Mol. Cell Res.** 1863: 1282-1297, 2016. (IF=4.521; 1)

9.31. Nejedlá M., Sadi S., Sulimenko V., de Almeida F.N., Blom H., Dráber P., Aspenström P, Karlsson R.: Profilin connects actin assembly with microtubule dynamics. **Mol. Biol. Cell** 27: 2381-2393, 2016. (IF=3.685; 12)

9.32. Sulimenko V., Hájková Z., Klebanovych A., Dráber P.: Regulation of microtubule nucleation mediated by γ-tubulin complexes. **Protoplasma** 254: 1187-1199, 2017. (IF=2.457; 6)

9.33. Dráberová E., Sulimenko V., Vinopal S., Sulimenko T., Sládková V., D'Agostino L., Sobol M., Hozák P., Křen L., Katsetos CD., Dráber P. Differential expression of human γ-tubulin isotypes during neuronal development and oxidative stress points to γ-tubulin 2 prosurvival function. **FASEB J.** 31: 1828-1848, 2017. (IF=5.595; 7)

9.34. Chumová J., Trögelová L., Kourová H., Volc J., Sulimenko V., Halada P., Kučera O., Benada O., Kuchařová A., Klebanovych A., Dráber P., Daniel G., Binarová P.: γ-Tubulin has a conserved intrinsic property of self-polymerization into double tranded filaments and fibrillar networks. **BBA Mol. Cell Res.** 1865: 734-748, 2018. (IF=4.739; 4)

9.35. Rubíková Z., Sulimenko V., Paulenda T., Dráber P.: Mast cell activation and microtubule organization are modulated by miltefosine through protein kinase C inhibition. **Front. Immunol.** 9: e1563, 2018. (IF=4.716)

9.36. Klebanovych A., Sládková V., Sulimenko, T., Vosecká V., Rubíková Z., Čapek M., Dráberová E., Dráber P., Sulimenko V.: Regulation of microtubule nucleation in mouse bone marrow-derived mast cells by protein tyrosine phosphatase SHP-1. **Cells** 8: e345, 2019. (IF=5.656)

9.37. Ivanova E., Gilet J.G., Sulimenko V., Duchon A., Rudolf G., Runge K., Collins S.C., Asselin L., Broix L., Drout N., Tilly, P., Nusbaum P., Vincent A., Magnant W., Skory V., Birling M.C., Pavlovic G., Godin J.D., Yalcin B., Hérault Y., Dráber P., Chelly J., Hinckelmann M.V.: *TUBG1* missense variants underlying cortical malformations disrupt neuronal locomotion and microtubule dynamics but not neurogenesis. **Nature Commun.** 10: e2129, 2019. (IF=11.878)