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Tubulin Posttranslational Modifications and Emerging Links to Human Disease

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Abstract

Tubulin posttranslational modifications are currently emerging as important regulators of the microtubule cytoskeleton and thus have a strong potential to be implicated in a number of disorders. Here we review the latest advances in understanding the physiological roles of tubulin modifications, and their link to a variety of pathologies.

Tubulin posttranslational modifications as regulators of microtubule functions

Microtubules are one of the major components of the eukaryotic cytoskeleton and carry out a wide variety of key functions in virtually every cell. Microtubules are essential for cell polarity, cell shape and intracellular transport. They build the mitotic and meiotic spindles, which are the key structures to divide chromosomes during cell division. Microtubules are also assembling specialized structures such as axonemes, which are the backbones of cilia and flagella, centrioles, the core structures of centrosomes, or marginal bands in blood platelets. Notwithstanding the multiple structures they build, microtubule filaments are always assembled from highly conserved dimers of α - and β -tubulin, which raises the question of how they are able to adapt to so many different functions.

In living cells, microtubules can interact with specific sets of microtubule-associated proteins (MAPs), including molecular motors and proteins that regulate microtubule dynamics. Thus, specialized microtubule functions depend to a large extent on which subset of MAPs they interact with. An emerging mechanism that can directly, and most likely selectively control these interactions are tubulin posttranslational modifications. Posttranslational modifications of tubulin have been known for many decades, however their functions have mostly remained elusive. This has changed over the last decade, when many of the enzymes involved in the catalysis of these modifications have been identified.

Mechanistic studies revealed that these modifications can selectively control the functions of microtubules by either directly controlling their mechanical properties, and thus, their stability, or by regulating the plethora of interactions with MAPs. These initial insights have underpinned the idea that tubulin modifications can form a kind of a ‘tubulin code’, which programs selected microtubules for specific intracellular functions (reviewed in Janke, 2014; Song and Brady, 2015)(cite snapshot here, Magiera et al.). Recently, the manipulation of tubulin-modifying enzymes in different model organisms has led to a significant advance in the understanding of the physiological contributions of these modifications and suggested links to pathologies. Moreover, the first direct links to human diseases have been identified with the discovery of mutations in genes encoding tubulin-modifying enzymes. Here we review the current state of understanding of tubulin posttranslational modifications in mouse models and human pathologies.

The essential role of tubulin modifications for cilia and flagella makes them risk factors for many cilia-related disorders

Most of the known tubulin modifications strongly accumulate at axonemes, the microtubule-based core structures of eukaryotic cilia and flagella. All functional data so far point to important roles of tubulin modifications in ciliary maintenance and function (Wloga et al., 2017). In humans, disorders of cilia can lead to a wide range of pathologies, commonly referred to as ciliopathies (Reiter and Leroux, 2017). These can be related to dysfunctions of motile cilia and flagella, but also to defects of the non-motile, primary cilia.

The beating of motile cilia and flagella is powered by axonemal dynein motors. Previous work identified tubulin polyglutamylation (addition of multiple glutamate residues to the C-terminal tubulin tails; Magiera et al. 2018) as an important regulator of this beating, and thus essential for ciliary function. Mutations in glutamylases (TTLL1, TTLL9) and deglutamylases (CCP1, CCP5) were linked to aberrations in spermatogenesis and sperm motility in mice, which resulted in male infertility (Konno et al., 2016; Mullen et al., 1976; Vogel et al., 2010; Wu et al., 2017). Alterations in tubulin polyglutamylation also affects the beating of other types of motile cilia, such as ependymal cilia in the brain ventricle (Bosch Grau et al., 2013), or airway cilia (Ikegami et al., 2010; Konno et al., 2016). While inactivation of the polyglutamylase TTLL1 leads to respiratory disorders in mice (Ikegami et al., 2010), no human disease directly linking aberrations of polyglutamylation to defects in motile cilia or sperm flagella has been identified so far.

Non-motile, or primary cilia, are found on many human cell types where they fulfill essential functions as sensory organelles and signaling hubs. In a mouse model with a mutation in the deglutamylase CCP1, upregulated polyglutamylation leads to degeneration of photoreceptors in the retina. Photoreceptors contain a modified form of primary cilia, the connecting cilium, which may become dysfunctional due to hyperglutamylation, and cause these cells to degenerate (Marchena et al., 2011). The importance of this mechanism for humans has been recently confirmed by the discovery of mutations in the tubulin deglutamylase CCP5 in human vision disorders (Astuti et al., 2016; Branham et al., 2016; Kastner et al., 2015).

Glycylation (addition of multiple glycine residues to the C-terminal tubulin tails, Magiera et al. 2018) is, in contrast to all other known tubulin modifications, predominantly found in cilia and flagella (Gadadhar et al., 2017; Wloga et al., 2017). Because of its specificity for cilia, glycylation is predestined to be a specific risk factor for ciliopathies. Tubulin glycylation is essential for the integrity of motile cilia in mouse ependymal cells (Bosch Grau et al., 2013), and for the control of primary cilia length (Gadadhar et al., 2017). Absence of glycylation in photoreceptors leads to shortening of the connecting cilium of these cells, which is followed

by retinal degeneration in mice (Bosch Grau et al., 2017). A rather unexpected link between glycylation and cancer was found in colorectal cancer. Knockout of the glycylyase *TTLL3* in mouse led to loss of primary cilia in colon and accelerated the proliferation of cancerous lesions. Strikingly, downregulation of *TTLL3* expression was linked to colon cancer in human patients, thus confirming the relevance of this mechanism for the human disease (Rocha et al., 2014).

Other tubulin modifications, such as detyrosination (removal of the ultimate gene-encoded tyrosine residue from α -tubulin), $\Delta 2$ -tubulin (removal of the penultimate glutamate from detyrosinated α -tubulin; (Johnson, 1998) and acetylation of the luminal lysine-40 residue of α -tubulin (Wloga et al., 2017; Magiera et al. 2018) are strongly enriched on axonemal microtubules of cilia and flagella. Among these, acetylation has been shown to have an effect on sperm morphology and motility as mice without the tubulin acetyl transferase α TAT1 are subfertile (Kalebic et al., 2013). Considering that human sperm is more prone to qualitative and quantitative deficiencies than their mouse counterparts, mutations that lead to subfertility in mice could potentially cause male sterility in human (Kherraf et al., 2017).

Perturbed tubulin modifications lead to neurodevelopmental and neurodegenerative disorders

Cytosolic microtubules are also subject to post-translational modification. Neurons present an interesting case as they are the only known cell type in which tubulin posttranslational modifications are strongly enriched on most cytosolic microtubules. The neuronal microtubule cytoskeleton is involved in neuronal differentiation and connectivity, and plays a key role in intracellular transport over long distances. Polyglutamylation accumulates during neuronal differentiation and is therefore considered a potential key regulator of these different functions of neuronal microtubules.

An important insight into the physiological role of polyglutamylation for neurons came with the discovery that a well-characterized mouse model for neurodegeneration, the Purkinje-cell degeneration (*pcd*) mouse (Mullen et al., 1976), bears a mutation in the gene encoding a deglutamylase enzyme, CCP1. The mutation leads to excessive accumulation of tubulin polyglutamylation specifically in regions of the nervous system that undergo degeneration (Rogowski et al., 2010). The most emblematic neurons that degenerate in the *pcd* mouse are Purkinje cells in the cerebellum and Mitral cells in the olfactory bulb (Mullen et al., 1976).

The timing of the degeneration of these two neuronal populations is different, which could be related to their individual susceptibilities to the deleterious insults.

The correlation between excessive polyglutamylation and neurodegeneration observed in *pcd* mice raises the possibility that many other neuron types could degenerate upon pathologically-upregulated tubulin polyglutamylation. This could be either induced by mutations in one of the modifying enzymes, or by defects in regulatory circuits that control the levels of this modification. So far, little is known about the molecular mechanisms that are controlled by polyglutamylation in neurons. Initial observations suggested an important role in the regulation of axonal transport with an impact on synaptic transmission (Ikegami et al., 2007; Maas et al., 2009), and it is also likely that it affects microtubule dynamics via the regulation of the microtubule-severing enzyme spastin (Lacroix et al., 2010; Valenstein and Roll-Mecak, 2016).

Detyrosination and $\Delta 2$ -tubulin are prominent modifications in neurons and found on all microtubules except those of the growth cone. Functional insight into the importance of these modifications came from tubulin-tyrosine ligase (TTL) knockout mice, which show strongly upregulated levels of detyrosinated and $\Delta 2$ -tubulin in the brain. TTL-knockout mice die perinatally due to massive defects in brain architecture, in particular in the cortico-thalamic loop (Erck et al., 2005). The most likely reasons for these defects are aberrant timing and extent of neurite outgrowth (Erck et al., 2005; Prota et al., 2013). Milder alterations of this modification could be involved in neurodevelopmental disorders in human, however no such cases have been found so far. The recent discovery of the enzymes catalyzing detyrosination (Aillaud et al., 2017; Nieuwenhuis et al., 2017) now allows a more thorough analysis of the enzymatic machinery that controls the detyrosination / tyrosination cycle and might thus point to disease links in humans.

Acetylation is enriched on all neuronal microtubules. During neuronal development, the modification controls axon branching (Wei et al., 2017) and plays a role in migration and morphological development of cortical neurons (Li et al., 2012). These functions could be explained by the recent finding that tubulin acetylation renders microtubules resistant to mechanical stress (Xu et al., 2017). However, given that acetylation is located in the inaccessible lumen of microtubules, the finding that this modification can also control axonal transport came as a surprise. Axonal transport defects have been linked to decreased tubulin acetylation in a range of neurodegenerative disorders, such as Huntington's disease (Dompierre et al., 2007), Charcot-Marie-Tooth disease (d'Ydewalle et al., 2011; Kim et al.,

2016), Amyotrophic lateral sclerosis (ALS) and Parkinson's disease (Godena et al., 2014). In these studies, which used mouse models or neurons obtained from patient-derived pluripotent stem cells, chemical inhibition of the deacetylating enzyme HDAC6 was sufficient to restore normal tubulin acetylation levels as well as normal axonal transport. To what extent acetylation alone is responsible for the observed transport defects remains to be verified, particularly because a mouse lacking tubulin acetyl transferase (ATAT1) merely showed defects in touch sensation but did not develop any of the expected degenerative phenotypes despite the complete absence of tubulin acetylation (Morley et al., 2016).

Modifications of platelet microtubules and their implications in bleeding disorders

Blood platelets are essential for the arrest of bleeding following injury, and their distinctive shape is maintained by a circular assembly of microtubules, the marginal band. Microtubules in the marginal band are rapidly reorganized during the process of platelet activation, which leads to blood clotting. The precise control of this peculiar microtubule assembly is thus an important factor in the function of platelets. Detyrosination, $\Delta 2$ -tubulin and acetylation have been detected on microtubules of marginal band (Diagouraga et al., 2014). Defects in tubulin acetylation affect the maturation of megakaryocytes (the precursors of platelets), and thus platelet formation (Iancu-Rubin et al., 2012). A tight control of acetylation and deacetylation events is further important during platelet spreading after activation (Sadoul et al., 2012). It is thus possible that defects in tubulin acetylation may play a role in human bleeding disorders.

Tubulin detyrosination in muscle functions and implications in cardiac diseases

Tubulin detyrosination accumulates at early steps of muscle cell differentiation, while acetylation levels rise later in this process. It has recently been demonstrated that tubulin detyrosination affects mechanotransduction in muscle cells (Kerr et al., 2015), and is important for load-bearing of buckling microtubules during cardiomyocyte contraction. Excess or diminished tubulin detyrosination changes the stiffness of the cardiomyocytes, thus leading to cardiac dysfunction. Upregulated tubulin detyrosination was found in patients diagnosed with hypertrophic and dilated cardiomyopathies (Robison et al., 2016). These first clinical links indicate that alterations of the tyrosination / detyrosination balance could be a risk factor for heart failure and be more generally linked to muscle dysfunctions in a range of different diseases.

Posttranslational modifications of tubulin in cell division control: possible implications for cancer

Most of the known tubulin modifications have been detected on microtubules of the cell division machinery, such as mitotic and meiotic spindles, midbody microtubules, and particularly, on centrioles, the microtubule-based core structures of the centrosomes. Tubulin modifications might control the precision of cell division; for instance, detyrosination, which impacts binding of a kinetochore-associated motor protein CENP-E, helps guide all chromosomes towards the metaphase plate in mitosis, and absence of this tubulin modification leads to misaligned chromosomes (Barisic et al., 2015). This deficit could lead to aneuploidy, a hallmark of cancer.

There are several indications that deregulated detyrosination is indeed linked to cancer. Differential expression of *TTL* correlates with poor prognosis in neuroblastoma tumors (Kato et al., 2004), and detyrosination of tubulin was particularly prominent in aggressive subtypes of breast cancer (Mialhe et al., 2001). Moreover, the recent discovery that vasohibins are the enzymes catalyzing detyrosination (Aillaud et al., 2017; Nieuwenhuis et al., 2017) provides new links between this tubulin modification and already known associations between vasohibin dysfunctions and cancer (Du et al., 2017).

In female meiosis, detyrosinated microtubules are asymmetrically enriched on one half of the meiotic spindle, which drives non-Mendelian chromosome transmission in mouse oocytes (Akeru et al., 2017). This shows that tubulin modifications can impact inheritance, and thus might affect the transmission of familial monogenic disorders, however no implication in a human pathology has so far been described.

Acetylation and polyglutamylation are both enriched on mitotic and meiotic spindle microtubules, midbody microtubules, and centrioles, however their functional contribution is yet to be elucidated. Injection of anti-glutamylation antibodies into dividing cells compromises centrosome integrity (Bobinnec et al., 1998), which suggests that the polyglutamylation of the centrioles is important for centrosome functions. Though no human disorder has been linked to tubulin modifications of centrioles, aberrations in these modifications might induce centrosome abnormalities, which are common hallmarks of cancer.

Conclusions and perspectives

Recent functional studies, most of them in mouse models, hint at a wide range of potential implications of tubulin posttranslational modifications in pathologies. Several predictions of disease-causing roles for tubulin modifications are now confirmed in human disorders, often with phenotypes highly similar to the ones observed in the mouse models. It is likely that the rapid advances in human genome profiling will uncover novel links between human pathologies and aberrant tubulin posttranslational modifications in the near future. Once these links are identified, it will be essential to determine how altered tubulin modifications contribute to the respective disease phenotypes. It will thus be necessary to further develop experimental systems that allow to directly measure the impact of tubulin modifications on microtubule properties and functions *in vitro* and in cell models. This could be challenging, as most of the known tubulin modifications appear to have rather subtle biological effects. While this might be one of the central reasons tubulin modifications have often been overlooked as regulators of biological processes, it also makes these modifications perfect candidates for risk factors of late-onset, or slow-developing disorders: In spite of being dispensable for basic functions of cells and organisms, fine-regulators might be important for their fitness and adaptability, and thus their resistance to potential disease-causing insults. It thus appears that a better understanding of the posttranslational regulation of the microtubule cytoskeleton might substantially change the way we perceive the implications of the cytoskeleton in human disease. As modifying enzymes are accessible targets for drug development (Huq and Wei, 2010), small-molecule inhibitors of tubulin-modifying enzymes are promising candidates for drugs for an emerging panoply of pathologies related to aberrant tubulin modifications.

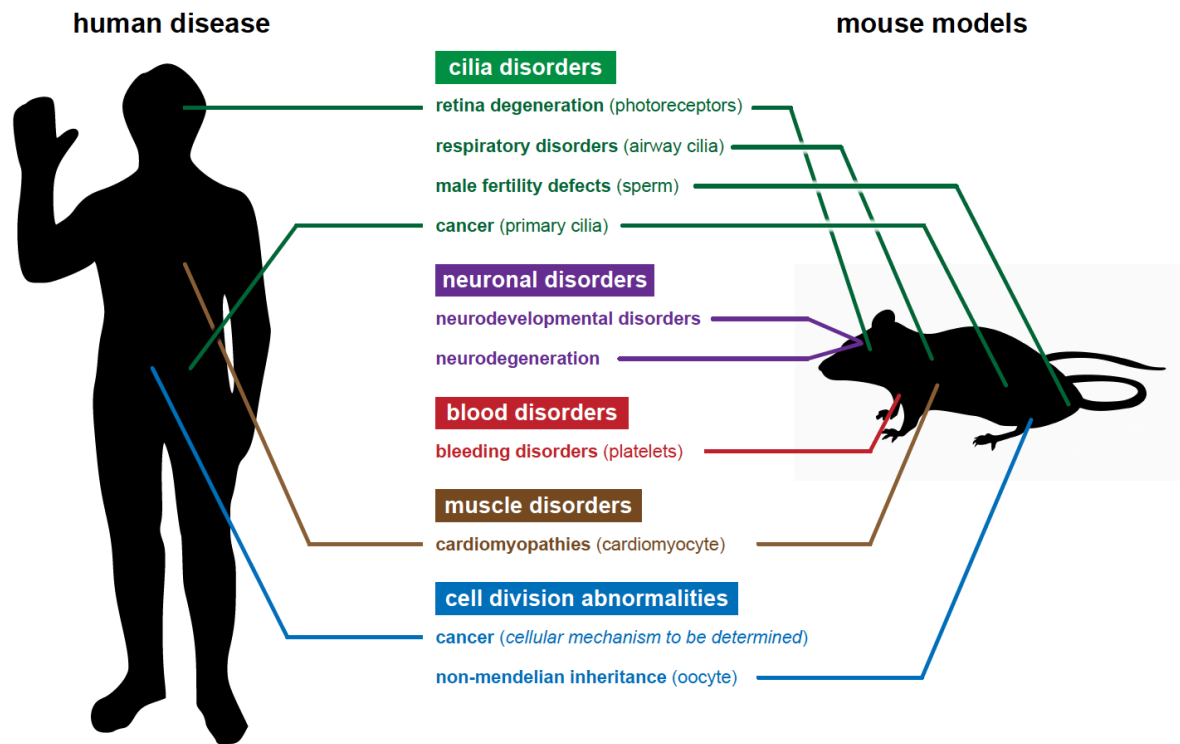


Figure 1: Known links between tubulin posttranslational modifications and disease pathologies

The known disorders that originate from aberrations of tubulin posttranslational modifications are listed, and their discovery in either mouse models or human patients is indicated.

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