Review

The tubulin code in mitosis and cancer

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Abstract: The “tubulin code” combines different α/β-tubulin isotypes with several post-translational modifications (PTMs) to generate microtubule diversity in cells. During cell division, specific microtubule populations in the mitotic spindle are differentially modified, but only recently the functional significance of the tubulin code, with particular emphasis on the role specified by tubulin PTMs, started to be elucidated. This is the case of α-tubulin detyrosination, which was shown to guide chromosomes during congression to the metaphase plate and allow the discrimination of mitotic errors, whose correction is required to prevent chromosomal instability - a hallmark of human cancers implicated in tumor evolution and metastasis. Although alterations in the expression of certain tubulin isotypes and associated PTMs have been reported in human cancers, it remains unclear whether and how the tubulin code has any functional implications for cancer cell properties. Here we review the role of the tubulin code in chromosome segregation during mitosis and how it impacts cancer cell properties. In this context, we discuss the existence of an emerging “cancer tubulin code” and the respective implications for diagnostic, prognostic and therapeutic purposes.

Keywords: cancer; chromosomal instability; microtubule; mitosis; tubulin code; tubulin post-translational modifications.

I. The tubulin code

Microtubules are dynamic, hollow cylindrical structures typically formed by thirteen laterally associated protofilaments of α/β-tubulin heterodimers that interact head-to-tail [1]. α- and β-tubulin proteins are encoded by several different genes (also known as tubulin isotypes) that diverge in their C-terminal tail regarding length and amino acid composition [2,3]. In eukaryotes, the expression and distribution of different tubulin isotypes is cell- and tissue-specific [2]. In addition, α- and β-tubulin isotypes may undergo multiple post-translational modifications (PTMs). As α/β-tubulin heterodimers polymerize into microtubules, the combination of isotype expression with PTMs generate microtubule diversity or a “tubulin code” (Figure 1), which has been implicated in the regulation of microtubule properties and functions underlying fundamental cellular processes [4,5].

Acetylation, detyrosination, polyglutamylation and polyglycylation are amongst the best characterized tubulin PTMs (Figure 1). Acetylation occurs in both α- and β-tubulins, more specifically at the luminal-side Lysine-40 (K40) of α-tubulin [6,7] and Lysine 252 (K252) of β-tubulin [8]. While K252 is modified by the acetyltransferase San [8], K40 is acetylated by the acetyltransferase MEC-17/αTAT1 [9,10] and deacetylated by histone deacetylase 6 (HDAC6) and sirtuin2 (SIRT2) [11,12]. When incorporated into microtubules, α-tubulin can also be detyrosinated, which consists on the catalytic removal of the last tyrosine present at the C-terminal tail of most isoforms by tubulin carboxypeptidases (TCPs), including the recently identified Vasohibin 1 (VASH1) and Vasohibin 2
(VASH2) complexes with their associated Small Vasohibin-Binding Protein (SVBP) [13-19]. As microtubules depolymerize, soluble detyrosinated α-tubulin can be retyrosinated by a highly specific tubulin tyrosine ligase (TTL) that closes the cycle [20,21]. Noteworthy, additional TCPs remain to be identified, as substantial α-tubulin detyrosination still occurs in human cells in which both Vasohibin-encoding genes were knocked out by CRISPR-Cas9 [14]. After detyrosination, α-tubulin C-terminal tails may also be subject to the removal of the penultimate and antepenultimate glutamates by cytosolic carboxypeptidases (CCPs) [22,23], leading to formation of the non-tyrosinatable Δ2- and Δ3-tubulin, respectively [24,25]. Additionally, C-terminal tails of both α- and β-tubulins undergo side-chain polyglutamylation and polyglycylation [26,27]. The single or consecutive addition of glutamate residues to the γ-carboxyl group of C-terminal tails is performed by several TTL-like (TTLL) (poly)glutamylases [5,28,29] and is/are removed by a set of CCPs known as deglutamylases [5,22,23,30]. Similarly, the addition of glycine residues relies on the (poly)glycylases TTLL3, TTLL8 and TTLL10 [31,32], but the identity of tubulin deglycylases remains unknown. Lastly, several other tubulin PTMs, such as methylation, polyamination, phosphorylation, ubiquitinylation, sumoylation, palmitoylation (reviewed in [5]) and O-GlcNAcylation [33] occur in the tubulin core structure adjacent to the C-terminal tails. These PTMs remain poorly characterized at the functional level but are likely to be implicated in microtubule assembly and dynamics [5,34,35].

**Figure 1.** The tubulin code combines different tubulin isotypes and PTMs to generate microtubule diversity. Only the best characterized isotypes and PTMs (+ respective enzymes) are depicted. See main text for details.

II. The tubulin code in mitosis
Mitosis relies on the critical contribution of microtubules, as well as several microtubule-associated proteins (MAPs) and motors, to regulate several key mechanisms underlying the faithful segregation of the genetic material during cell division. It involves the assembly of a specialized microtubule-based structure known as the mitotic spindle. Due to their intrinsic dynamic nature, mitotic spindle microtubules are vastly tyrosinated, i.e. remain essentially non-modified (note that most gene-encoded α-tubulin isoforms carry a last Tyrosine residue at their C-terminal tails; see Figure 1). As some spindle microtubules become gradually stabilized due to the establishment of chromosome attachments at the kinetochore, as well as possible interactions between some interpolar microtubules, they become increasingly detyrosinated [19,36-40] (Figure 2). Likewise, kinetochore microtubules are highly acetylated on K40 of α-tubulin [36,41], polyglutamylated [42], and accumulate Δ2-tubulin [43]. The action of spindle microtubules during mitosis is regulated by several MAPs [44] and assisted by several motor proteins [45]. For instance, the initial capture and transport of peripheral chromosomes by microtubules is mediated by dynein/dynactin [46-49], a minus-end-directed motor localized at unattached kinetochores [50,51], whereas the subsequent congression to the spindle equator is mediated by another kinetochore-associated motor, Centromere Protein E (CENP-E)/kinesin-7, with microtubule plus-end-directed activity [52,53]. Other mitotic motors include kinesin-5, which slides antiparallel microtubules to ensure proper centrosome separation, spindle bipolarity and spindle elongation during anaphase, as well as kinesin-13s, which lack motor activity but promote microtubule depolymerization to control spindle length and mediate mitotic error correction [54-59]. Thus, the mitotic spindle is an anisotropic and highly heterogeneous structure, with dynamic astral microtubules essentially tyrosinated, in contrast with more stable microtubule subpopulations, such as kinetochore and a fraction of interpolar microtubules, which accumulate detyrosinated, Δ2, acetylated and polyglutamylated tubulin. How these modifications impact the action of the different mitotic motors that assist chromosome segregation remains poorly understood.

- A navigation system guides chromosomes to the spindle equator

Although tubulin diversity in the mitotic spindle has been recognized for several decades, the respective functional relevance for mitosis remained unclear until recently. One crucial implication of the tubulin code hypothesis is the regulation of MAPs and motors by specific tubulin isotypes and PTMs [4]. Original work in neurons revealed that classic kinesin motors, such as Kinesin-I, are able to recognize and have a preference for microtubules with particular tubulin PTMs, namely detyrosination and acetylation [60,61]. Subsequently, α-tubulin detyrosination was shown to regulate mitotic chromosome congression to the metaphase plate by guiding the microtubule plus-end-directed motor CENP-E/kinesin-7 at kinetochores in human cells [36]. In contrast, the microtubule minus-end-directed motor dynein/dynactin that is also localized at unattached kinetochores [51,62], preferentially associates with tyrosinated microtubules [40,63-65], which favors the initiation of motion, but is dispensable for subsequent dynein/dynactin processivity [64,65]. Thus, detyrosinated/tyrosinated α-tubulin regulates the activity of opposing kinetochore motors, establishing a navigation system for chromosomes that assists their congression to the spindle equator [66] (Figure 2). Accordingly, during the initial capture of chromosomes, dynein/dynactin counteracts the action of chromokinesins on chromosome arms to move peripheral chromosomes along tyrosinated astral microtubules towards the vicinity of the poles [67]. By transporting peripheral chromosomes to the poles where the microtubule destabilizing activity of Aurora A kinase is higher [68,69], dynein/dynactin prevents the formation of stable end-on kinetochore–microtubule attachments that would otherwise cause the random ejection of polar chromosomes by chromokinesins [66,67]. Once at the poles, Aurora A-mediated phosphorylation activates CENP-E at kinetochores of polar chromosomes [70], thus allowing their transport specifically along detyrosinated spindle microtubules towards the equator. In agreement, recent super-resolution Coherent-Hybrid Stimulated Emission Depletion microscopy [71] of CENP-E-GFP revealed its exclusive association with stable kinetochore- and interpolar microtubule bundles, but not with tyrosinated astral microtubules [72]. Curiously, α-tubulin acetylation on K40, which is also enriched
on stable spindle microtubules [41], does not interfere with polar chromosome congression [36]. While the potential contribution of other tubulin PTMs to chromosome congression remains unknown, these findings support a robust working model in which tyrosinated/detyrosinated microtubules guide peripheral chromosomes towards the spindle equator.

- **A mitotic error code**

  Regulation of kinetochore microtubule dynamics is essential for error correction and the maintenance of genome stability since it allows the establishment of *amphitelic* kinetochore-MT attachments that lead to chromosome bi-orientation relative to the spindle poles. Kinesin-13s, such as Kinesin superfamily 2b (Kif2b) and Mitotic Centromere Associated Kinesin (MCAK), promote kinetochore microtubule dynamics, thus playing a key role in the correction of mal-oriented chromosomes with erroneous kinetochore-microtubule attachments (e.g. syntelic in which both sister kinetochores are oriented towards a single spindle pole, and merotelic where a single kinetochore is attached with microtubules oriented to both poles) and ultimately in the prevention of chromosome missegregation [55,73] (Figure 2). In agreement, stimulation of kinetochore microtubule dynamics in otherwise chromosomally unstable cancer cells by increasing Kinesin-13 depolymerase activity reestablished chromosomal stability [55,74]. Building on the previous finding that MCAK’s microtubule depolymerizing activity is reduced four fold in the presence of detyrosinated microtubules *in vitro* [75,76], it was recently shown that the mitotic error correction activity of MCAK and Kif2b is regulated by α-tubulin detyrosination [37]. Accordingly, experimental depletion of TTL or overexpression of VASH1/SVBP, which caused a constitutive increase of α-tubulin detyrosination in the vicinity of the kinetochores, compromised error correction, leading to chromosome segregation errors. Importantly, α-tubulin detyrosination specifically impaired the MCAK-based error correction machinery located on centromeres/kinetochores and it did so without affecting global kinetochore microtubule dynamics, suggesting that mitotic error correction is exquisitely sensitive to the detyrosinated state of α-tubulin that likely occurs at the individual microtubule level. These data support the existence of a “mitotic error code” in which α-tubulin detyrosination/tyrosination signals and regulates MCAK activity at centromeres/kinetochores to discriminate between correct and incorrect kinetochore-MT attachments during mitosis (Figure 2).

  Complete centrosome separation before nuclear envelope breakdown prevents subsequent segregation errors and ensures mitotic fidelity [77]. This relies on several elements, including the microtubule motors kinesin-5, required for centrosome separation, and dynein/dynactin, which promotes both centrosome separation and positioning [78,79]. Similar to dynein/dynactin, kinesin-5 appears to have increased affinity to tyrosinated dendritic microtubules in neurons [80], but direct evidence from *in vitro* reconstitution assays is still lacking. Nonetheless, recent work in which centrosome positioning in human mitotic cells was tracked in 3D indicated that centrosome separation at nuclear envelope breakdown is insensitive to the tyrosinated state of α-tubulin [37]. This reinforces the idea that the observed increase in mitotic errors associated with excessive α-tubulin detyrosination is due to the incapacity to correct, rather than an increased propensity to make errors.

- **Role in mitotic spindle orientation and positioning**

  Mitotic spindle orientation and positioning in the cell center is essential for accurate cell division and relies on the action of pulling forces on astral microtubules [81]. In particular, dynein/dynactin anchored to cortical proteins or cytoplasmic organelles was shown to play a significant role in spindle orientation/positioning [82-84], possibly through its increased affinity to tyrosinated astral microtubules (Figure 1). Indeed, modulation of α-tubulin tyrosination state, either through TTL knockout [40] or CRISPR/Cas9-mediated editing of the C-terminal tyrosine [84], caused spindle orientation defects. In contrast, experimental decrease of α-tubulin detyrosination after VASH1/2 silencing increased the depolymerase activity of MCAK, resulting in disoriented spindles, with shorter astral microtubules [19]. Taken together, these observations indicate that the mechanisms
behind spindle orientation/positioning rely on the intrinsic nature (i.e. non-modified) of tyrosinated α-tubulin to allow astral microtubules to establish a correct cell division plane (Figure 2).

Figure 2. Summary of the established roles of the tubulin code in mitosis. The initial capture of peripheral chromosomes is mediated by Dynein/Dynactin at kinetochores, upon which the chromosome is brought to the vicinity of the centrosome by lateral transport along tyrosinated astral microtubules. This prevents the random ejection of the chromosome by the action of Chromokinesins on chromosome arms. Once at the pole, high Aurora A activity prevents stabilization of end-on kinetochore-microtubule attachments, which otherwise would favor the action of Chromokinesins on chromosome arms. In parallel, Aurora A-mediated phosphorylation activates CENP-E at kinetochores. This initiates transport towards the spindle equator (congregation) along stable detyrosinated microtubules. MCAK and Kif2b (not depicted) at centromeres and kinetochores are also inhibited by tubulin detyrosination on kinetochore microtubules, allowing the correction of syntelic and merotelic attachments, while preserving correct amphitelic attachments on bi-oriented chromosomes. MCAK at microtubule plus ends also regulates astral microtubule length to allow interaction with Dynein/Dynactin at the cortex or cytoplasmic organelles (not depicted), which exerts pulling forces necessary for spindle orientation and positioning. See main text for details.

- **Roles in centrosome structure and cytokinesis**

  Tubulin polyglutamylation is highly enriched on centriole microtubules [42,85] and has been proposed to contribute to normal mitosis by maintaining centrosome structure [85,86]. Indeed, recent
super-resolution imaging of centriole structure revealed the specific distribution of polyglutamylation on centriole MTs and suggested a key role for this PTM in ultrastructural organization of specific centriolar proteins [87]. Furthermore, tubulin polyglutamylation promotes the activity of the microtubule severing enzymes spastin and katanin [88-91], which are also implicated in cell division. Indeed, their activities regulate several cellular processes that likely impact chromosome segregation fidelity, such as microtubule poleward flux, spindle orientation and length [92-94]. Spastin and katanin are also required for the abscission step and completion of cytokinesis [95-97]. Like spastin [95] and katanin [97], polyglutamylated tubulin is enriched at the midbody [88], and a tubulin mutation that compromises polyglutamylation (and possibly also polyglycylation) in cilia was shown to cause cytokinesis defects [98]. These results suggest that the completion of cytokinesis relies on the regulation of spastin and katanin activities by tubulin polyglutamylation.

III. The cancer tubulin code

- (De)regulation of tubulin isotypes and PTMs in cancer

Several works have reported an emerging link between alterations of tubulin isotypes and PTMs and/or associated modifying enzymes with certain cancers, most noticeable those occurring in breast, colon, prostate, liver, brain, bile duct and pancreas (Table 1). These alterations often correlate with specific cancer properties, including poor outcome/prognosis [99-101] and metastatic ability [99], supporting the potential use of cancer tubulin isotypes and/or PTM signatures as useful biomarkers, as well as for therapeutic purposes. However, a comprehensive and definitive view on the real potential is still lacking, especially concerning causality, since the available data is still limited and often contradictory.

| Table 1. Tubulin isotypes, post-translational modifications and modifying enzymes in cancer |
|-----------------------------------------------|---------------------------------|------------------|------------------|
| Tubulin PTM (and/or enzymes)/ Isotype         | Cancer                         | Regulation       | References       |
| Detyrosination                                | Prostate Cancer Cells          | Up-regulated     | [102]            |
|                                               | Poor Prognosis Breast Tumors   | Up-regulated     | [100]            |
|                                               | Invasive Ductal Carcinoma      | Up-regulated     | [103]            |
|                                               | (Breast)                       |                  |                  |
| TTL                                           | Prostate Cancer Cells          | Down-regulated   | [102]            |
|                                               | Poor Prognosis Neuroblastomas  | Down-regulated   | [101]            |
| VASH2                                         | Hepatocellular carcinoma       | Up-regulated     | [104]            |
|                                               | Tissues and Cell Lines         |                  |                  |
| Δ2-Tubulin                                    | Prostate Cancer Cells          | Down-regulated   | [102]            |
| Acetylation                                   | Metastatic Breast Tumors and   | Up-regulated     | [99]             |
|                                               | Cell Lines                     |                  |                  |
|                                               | Pancreatic Tumors              | Up-regulated     | [105]            |
|                                               | Glioblastoma Tissues and Cell  | Up-regulated     | [106]            |
|                                               | Lines                          |                  |                  |
|                                               | Cholangiocarcinoma Cell Lines  | Up-regulated     | [107]            |
| Glutamylation/Polyglutamylation               | Prostate Cancer Cells          | Up-regulated     | [102]            |
ubulin detyrosination in cancer

Accordingly, the expression of HDAC6 promotes angiogenesis, as evidenced in experiments involving the administration of ectopic VASH1, which appears to play an important role in cancer cell proliferation, and suggests a cancer suppressing role for VASH1 and VASH2.

The recent discovery of Vasohibins (VASH1 and VASH2) as TCPs [13,14] revitalized the discussion about the role of tubulin detyrosination in cancer. Vasohibins and their associated SVBP were originally identified as secreted proteins implicated in angiogenesis [128]. While VASH2 promotes vascularity by accumulating at the sprouting zone, VASH1 expression is increased in endothelial cells of the termination zone, where it inhibits vascularity [129]. During tumor development in mice xenograft models, experiments involving administration of ectopic VASH1 indicated that it inhibits tumor lymphangiogenesis [130], angiogenesis and growth [131]. On the other hand, VASH2, which appears to play an important role in cancer cell proliferation [104], promotes tumor angiogenesis and growth [104,132-134]. Noteworthy, none of these studies demonstrate that the observed impact in cancer was due to defective tubulin detyrosination. However, a recent work...
reported that human patients suffering from a broad range of carcinomas had mutations in VASH1 and VASH2 that compromised their tubulin detyrosination activity [17]. Taken together, these findings suggest that in addition to downregulation of TTL [127], the link between tubulin detyrosination and tumorigenesis may be attributed to the increased expression of Vasohibins. The availability of VASH1/2-SVBP knockout mice [129,135] will be instrumental to clarify the apparently opposite roles of VASH1 and VASH2 in cancer and whether this is due to their secreted and/or tubulin detyrosinating activities.

Figure 3. Implications of the tubulin code for tumor progression and metastasis. While downregulation of TTLL3 (glycylation), together with the expression of VASH2 (detyrosination), HDAC6 (acetylation) and β3-tubulin promote tumor growth, this is inhibited by VASH1 (detyrosination). Tumor formation and chromosomal instability is also associated with the downregulation of TTL. Tubulin acetylation, detyrosination and β3-tubulin isotype might promote several steps of metastasis associated with the epithelial-to-mesenchymal transition, such as cell migration and invasion. See main text for details.

- **The cancer tubulin code in cell migration and invasion**

Tubulin PTMs have also been implicated in epithelial-to-mesenchymal transition (EMT), a key process behind metastasis initiation. For instance, experimental increase of the tubulin deacetylase HDAC6 promoted EMT, whereas TGF-β induction of EMT downregulated tubulin acetylation [136]. Likewise, the induction of EMT also correlated with downregulation of TTL and the consequent increase of tubulin detyrosination [103], as shown before during tumor development [127], thus pointing to the possible involvement of these tubulin PTMs and associated enzymes in cell transformation.

Interestingly, tubulin acetylation is also frequently associated with the regulation of cell migration, although this remains controversial. While HDAC6 expression and activity was proposed to promote cell migration [12,126,136-138], the opposite effect was observed after the loss of αTat1 or mutation of the α-tubulin lysine 40 (K40R) [99,137,139,140]. The establishment of cell adhesion to the substrate also has implications for cell motility, and the loss of either HDAC6 or αTat1 leads to an
increased focal adhesion area and number, respectively, as well as decreased dynamics [137,141]. However, other works reported that loss of αTat1 leads to a decrease in focal adhesion number [122]. The basis for this discrepancy remains unclear, but it is likely associated with different experimental set ups: one study investigated the role of αTat1 in wound-induced migrating cells [137], while the other used normally growing cells were used [122], raising the possibility that αTat1 promotes focal adhesion dynamics specifically during cell migration.

The upregulation of tubulin acetylation in metastatic breast tumors and cell lines [99] is consistent with its association with cancer cell invasiveness. RNAi-mediated depletion of either αTat1 or HDAC6 indicated that their expression induced breast cancer cell invasion [139,140,142]. Additionally, the increased tubulin acetylation of these metastatic breast cancer cells promoted microtentacle generation and cell reattachment ability, essential for metastasis [99]. Likewise, a high frequency of microtentacles and cell reattachment was also associated with tubulin detyrosination [103,143]. Collectively, these data favor a potential role of tubulin acetylation in metastasis progression. While HDAC6 indiscriminately acts upon multiple protein targets, the direct modulation of tubulin acetylation by K40R mutation experiments suggest that the upregulation of tubulin acetylation is a metastasis-promoting factor, supporting the αTat1-related findings. This would explain the link between upregulation of tubulin acetylation and poor prognosis in breast cancer patients [99], but unspecified effects due to overexpression of GFP-tagged K40R mutant α-tubulin cannot be excluded.

IV. How alterations of the tubulin code in mitosis might be implicated in cancer

Chromosomal instability, a hallmark of cancers, has been shown to promote the metastatic process [74]. Indeed, the overexpression of Kif2b or MCAK, in addition to reestablishing the stability of chromosomally unstable cancer cells [55,74], inhibits metastasis in vitro and in vivo, with a consequent increase in survival [74]. Given that excessive tubulin detyrosination might lead to chromosomal instability by suppressing the error correction activity of MCAK and Kif2b [37], together with the observed upregulation of tubulin detyrosination in invasive cancer and with poor prognosis (Table 1), it raises the exciting possibility that an increase in tubulin detyrosination might promote cancer progression through inhibition of the mitotic error correction machinery. However, an extensive analysis of tubulin detyrosination in chromosomal instability-prone cancers, together with the elucidation of its implications for cancer metastasis, is necessary for its establishment as potential diagnostic and prognostic biomarkers. In addition, tubulin detyrosination represents a promising therapeutic target for cancer suppression, for example by using TCP inhibitors, such as epoY [13] or parthenolide [144].

Deregulation of tubulin detyrosination in cancers might also be involved in other mitotic-related cancer features. Firstly, the cell cycle delay observed upon VASH1/2 [19] and VASH2 [104] deletion might unveil the importance of VASH2 for proper cancer cell proliferation and tumor development [104,132-134]. Furthermore, both experimental upregulation and downregulation of tubulin detyrosination led to congression defects, causing alterations in CENP-E-mediated transport of chromosomes to the spindle equator [36]. Additionally, the decrease of CENP-E expression is well established to promote mild chromosomal instability and aneuploidy, as well as tumorigenesis in mice [145-148]. Therefore, deregulation of tubulin detyrosination in cancers (Table 1) may also account for cancer promotion under conditions of moderate chromosomal instability, such as those associated with mild problems in chromosome congression. Further investigation is required to fully understand the potential implications of tubulin detyrosination and other PTMs for tumorigenesis and the respective link with chromosomal instability.

V. Conclusions and outlook

Since the initial observations implicating tubulin PTMs in cell division, recent works have allowed a deeper understanding of their involvement, in particular detyrosination/tyrosination, in the coordination of several mechanisms underlying faithful chromosome segregation during mitosis. However, considerable knowledge is still lacking in order to establish a complete picture of the roles
played by the tubulin code in mitosis. The scenario is no different regarding the emerging cancer tubulin code, in which a considerable amount of disconnected data dominates. Nevertheless, there are already some promising links between deregulation of certain tubulin isotypes and PTMs (notoriously acetylation, detyrosination and glycylation) and several cancers. A more systematic investigation of these links will be of high priority to the field and might prove important for diagnostic and prognostic purposes. This is likely to have a major impact in understanding and mitigating acquired resistance to microtubule-targeting drugs, the biggest threat in current cancer chemotherapy. Future work is also necessary to establish clear functional links beyond correlations by taking advantage of emerging molecular tools and model systems for modulation and analysis of tubulin isotypes and PTMs (both in vitro and in vivo) that will strengthen and clarify their potential therapeutic value for the treatment of human cancers.

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**References**

1. Desai, A.; Mitchison, T.J. Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* 1997, 13, 83-117.
2. Ludueña, R.F.; Banerjee, A. The isotypes of tubulin. In *The role of microtubules in cell biology, neurobiology, and oncology*, Springer: 2008; pp 123-175.
3. Janke, C. The tubulin code: Molecular components, readout mechanisms, and functions. *J Cell Biol* 2014, 206, 461-472.
4. Verhey, K.J.; Gaertig, J. The tubulin code. *Cell Cycle* 2007, 6, 2152-2160.
5. Janke, C.; Magiera, M.M. The tubulin code and its role in controlling microtubule properties and functions. *Nat Rev Mol Cell Biol* 2020, 21, 307-326.
6. L’Hernault, S.W.; Rosenbaum, J.L. Chlamydomonas alpha-tubulin is posttranslationally modified by acetylation on the epsilon-amino group of a lysine. *Biochemistry* 1985, 24, 473-478.
7. Soppina, V.; Herbstman, J.F.; Skiniotis, G.; Verhey, K.J. Luminal localization of alpha-tubulin k40 acetylation by cryo-em analysis of fab-labeled microtubules. *PloS one* 2012, 7.
8. Chu, C.W.; Hou, F.; Zhang, J.; Phu, L.; Loktev, A.V.; Kirkpatrick, D.S.; Jackson, P.K.; Zhao, Y.; Zou, H. A novel acetylation of beta-tubulin by san modulates microtubule polymerization via down-regulating tubulin incorporation. *Mol Biol Cell* 2011, 22, 448-456.
9. Akella, J.S.; Wloga, D.; Kim, J.; Starostina, N.G.; Lyons-Abbott, S.; Morrissette, N.S.; Dougan, S.T.; Kipreos, E.T.; Gaertig, J. Mec-17 is an alpha-tubulin acetyltransferase. *Nature* 2010, 467, 218-222.
10. Shida, T.; Cueva, J.G.; Xu, Z.; Goodman, M.B.; Nachury, M.V. The major alpha-tubulin k40 acetyltransferase alphata1 promotes rapid ciliogenesis and efficient mechanosensation. *Proceedings of the National Academy of Sciences of the United States of America* 2010, 107, 21517-21522.
11. North, B.J.; Marshall, B.L.; Borra, M.T.; Denu, J.M.; Verdin, E. The human sir2 ortholog, sirt2, is an nad+-dependent tubulin deacetylase. *Molecular cell* 2003, 11, 437-444.
12. Hubbert, C.; Guardiola, A.; Shao, R.; Kawaguchi, Y.; Ito, A.; Nixon, A.; Yoshida, M.; Wang, X.F.; Yao, T.P. Hdac6 is a microtubule-associated deacetylase. *Nature* 2002, 417, 455-458.
13. Aillaud, C.; Bosc, C.; Peris, L.; Bosson, A.; Heemeryck, P.; Van Dijk, J.; Le Friec, J.; Boulan, B.; Vossier, F.; Sanman, L.E., et al. Vasohibins/svbp are tubulin carboxypeptidases (tcps) that regulate neuron differentiation. Science 2017, 358, 1448-1453.

14. Nieuwenhuis, J.; Adamopoulos, A.; Bleijerveld, O.B.; Mazouzi, A.; Stickel, E.; Celie, P.; Altelaar, M.; Knipscheer, P.; Perrakis, A.; Blomen, V.A., et al. Vasohibins encode tubulin detyrosinating activity. Science (New York, N.Y) 2017, 358, 1453-1456.

15. Li, F.; Li, Y.; Ye, X.; Gao, H.; Shi, Z.; Luo, X.; Rice, L.M.; Yu, H. Cryo-em structure of vash1-svbp bound to microtubules. Elife 2020, 9.

16. Liu, X.; Wang, H.; Zhu, J.; Xie, Y.; Liang, X.; Chen, Z.; Feng, Y.; Zhang, Y. Structural insights into tubulin detyrosination by vasohibins-svbp complex. Cell discovery 2019, 5, 65.

17. Wang, N.; Bosc, C.; Ryul Choi, S.; Boulan, B.; Peris, L.; Olieric, N.; Bao, H.; Krichen, F.; Chen, L.; Andrieux, A., et al. Structural basis of tubulin detyrosination by the vasohibin-svbp enzyme complex. Nat Struct Mol Biol 2019, 26, 571-582.

18. Li, F.; Hu, Y.; Qi, S.; Luo, X.; Yu, H. Structural basis of tubulin detyrosination by vasohibins. Nat Struct Mol Biol 2019, 26, 583-591.

19. Liao, S.; Rajendraprasad, G.; Wang, N.; Eibes, S.; Gao, J.; Yu, H.; Wu, G.; Tu, X.; Huang, H.; Barisic, M., et al. Molecular basis of vasohibins-mediated detyrosination and its impact on spindle function and mitosis. Cell Res 2019, 29, 533-547.

20. Ersfeld, K.; Wehland, J.; Plessmann, U.; Dodemont, H.; Gerke, V.; Weber, K. Characterization of the tubulin-tyrosine ligase. J Cell Biol 1993, 120, 725-732.

21. Schroder, H.C.; Wehland, J.; Weber, K. Purification of brain tubulin-tyrosine ligase by biochemical and immunological methods. The Journal of cell biology 1985, 100, 276-281.

22. Rogowski, K.; van Dijk, J.; Magiera, M.M.; Bosc, C.; Deloulme, J.C.; Bosson, A.; Peris, L.; Gold, N.D.; Lacroix, B.; Bosch Grau, M., et al. A family of protein-deglutamylating enzymes associated with neurodegeneration. Cell 2010, 143, 564-578.

23. Tort, O.; Tanco, S.; Rocha, C.; Bieche, I.; Seixas, C.; Bosc, C.; Andrieux, A.; Moutin, M.J.; Aviles, F.X.; Lorenzo, J., et al. The cytosolic carboxypeptidases ccp2 and ccp3 catalyze posttranslational removal of acidic amino acids. Mol Biol Cell 2014, 25, 3017-3027.

24. Paturle-Lafanecere, L.; Edde, B.; Denoulet, P.; Van Dorsseelaer, A.; Mazarguil, H.; Le Caer, J.P.; Wehland, J.; Job, D. Characterization of a major brain tubulin variant which cannot be tyrosinated. Biochemistry 1991, 30, 10523-10528.

25. Edde, B.; Rossier, J.; Le Caer, J.P.; Desbruyeres, E.; Gros, F.; Denoulet, P. Posttranslational glutamylation of alpha-tubulin. Science 1990, 247, 83-85.

26. Redeker, V.; Levilliers, N.; Schmitter, J.M.; Le Caer, J.P.; Rossier, J.; Adoutte, A.; Bre, M.H. Polyglycylation of tubulin: A posttranslational modification in axonemal microtubules. Science 1994, 266, 1688-1691.

27. Janke, C.; Rogowski, K.; Wloka, D.; Regnard, C.; Kajava, A.V.; Strub, J.M.; Temurak, N.; van Dijk, J.; Boucher, D.; van Dorsseelaer, A., et al. Tubulin polyglutamylase enzymes are members of the ttl domain protein family. Science 2005, 308, 1758-1762.
29. van Dijk, J.; Rogowski, K.; Miro, J.; Lacroix, B.; Edde, B.; Janke, C. A targeted multienzyme mechanism for selective microtubule polyglutamylation. *Mol Cell* 2007, 26, 437-448.

30. Kimura, Y.; Kurabe, N.; Ikekami, K.; Tsutsumi, K.; Konishi, Y.; Kaplan, O.I.; Kunitomo, H.; Iino, Y.; Blacque, O.E.; Setou, M. Identification of tubulin de glutamylase among caenorhabditis elegans and mammalian cytosolic carboxypeptidases (ccps). *The Journal of Biological Chemistry* 2010, 285, 22936-22941.

31. Rogowski, K.; Juge, F.; van Dijk, J.; Wloga, D.; Strub, J.M.; Levilliers, N.; Thomas, D.; Bre, M.H.; Van Dorsselaer, A.; Gaertig, J., et al. Evolutionary divergence of enzymatic mechanisms for posttranslational polyglycylation. *Cell* 2009, 137, 1076-1087.

32. Wloga, D.; Webster, D.M.; Rogowski, K.; Bre, M.H.; Levilliers, N.; Jerka-Dziadosz, M.; Janke, C.; Dougan, S.T.; Gaertig, J. Tll3 is a tubulin glycine ligase that regulates the assembly of cilia. *Developmental Cell* 2009, 16, 867-876.

33. Walgren, J.L.; Vincent, T.S.; Schey, K.L.; Buse, M.G. High glucose and insulin promote o-glcnac modification of proteins, including alpha-tubulin. *American Journal of Physiology. Endocrinology and Metabolism* 2003, 284, E424-E434.

34. Tian, J.L.; Qin, H. O-glcnacylation regulates primary ciliary length by promoting microtubule disassembly. *iScience* 2019, 12, 379-391.

35. Ji, S.; Kang, J.G.; Park, S.Y.; Lee, J.; Oh, Y.J.; Cho, J.W. O-glcnacylation of tubulin inhibits its polymerization. *Amino Acids* 2011, 40, 809-818.

36. Barisic, M.; Silva e Sousa, R.; Tripathy, S.K.; Magiera, M.M.; Zaytsev, A.V.; Pereira, A.L.; Janke, C.; Grischchuk, E.L.; Maiato, H. Mitosis. Microtubule detyrosination guides chromosomes during mitosis. *Science* 2015, 348, 799-803.

37. Ferreira, L.T.; Orr, B.; Rajendraprasad, G.; Pereira, A.J.; Lemos, C.; Lima, J.T.; Guasch Boldu, C.; Ferreira, J.G.; Barisic, M.; Maiato, H. Alpha-tubulin detyrosination impairs mitotic error correction by suppressing mcaK centromeric activity. *The Journal of Cell Biology* 2020, 219.

38. Gundersen, G.G.; Bulinski, J.C. Distribution of tyrosinated and nontyrosinated alpha-tubulin during mitosis. *J Cell Biol* 1986, 102, 1118-1126.

39. Gundersen, G.G.; Kalnosski, M.H.; Bulinski, J.C. Distinct populations of microtubules: Tyrosinated and nontyrosinated alpha tubulin are distributed differently in vivo. *Cell* 1984, 38, 779-789.

40. Peris, L.; Thery, M.; Faure, J.; Saoudi, Y.; Lafanechere, L.; Chilton, J.K.; Gordon-Weeks, P.; Galjart, N.; Bornens, M.; Wordeman, L., et al. Tubulin tyrosination is a major factor affecting the recruitment of captgly proteins at microtubule plus ends. *J Cell Biol* 2006, 174, 839-849.

41. Wilson, P.J.; Forer, A. Acetylated alpha-tubulin in spermatogenic cells of the crane fly neptrotoma sutturalis - kinetochore microtubules are selectively acetylated. *Cell Motility and the Cytoskeleton* 1989, 14, 237-250.

42. Bobiniec, Y.; Moudjou, M.; Fouquet, J.P.; Desbruieres, E.; Edde, B.; Bornens, M. Glutamylation of centriole and cytoplasmic tubulin in proliferating non-neuronal cells. *Cell Motil Cytoskeleton* 1998, 39, 223-232.

43. Ferreira, L.T.; Figueiredo, A.C.; Orr, B.; Lopes, D.; Maiato, H. Dissecting the role of the tubulin code in mitosis. In *Methods in Cell Biology*, Elsevier: 2018; Vol. 144, pp 33-74.

44. Maiato, H.; Sampaio, P.; Sunkel, C.E. Microtubule-associated proteins and their essential roles during mitosis. *Int Rev Cytol* 2004, 241, 53-153.

45. Cross, R.A.; McAinsh, A. Prime movers: The mechanochemistry of mitotic kinesins. *Nature Reviews* 2014, 15, 257-271.
46. Yang, Z.; Tulu, U.S.; Wadsworth, P.; Rieder, C.L. Kinetochore dynein is required for chromosome motion and congression independent of the spindle checkpoint. *Curr Biol* 2007, 17, 973-980.

47. Hayden, J.H.; Bowser, S.S.; Rieder, C.L. Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: Direct visualization in live newt lung cells. *The Journal of cell biology* 1990, 111, 1039-1045.

48. Li, Y.; Yu, W.; Liang, Y.; Zhu, X. Kinetochore dynein generates a poleward pulling force to facilitate congression and full chromosome alignment. *Cell Res* 2007, 17, 701-712.

49. Vorozhko, V.V.; Emanuele, M.J.; Kallio, M.J.; Stukenberg, P.T.; Gorbsky, G.J. Multiple mechanisms of chromosome movement in vertebrate cells mediated through the ndc80 complex and dynein/dynactin. *Chromosoma* 2008, 117, 169-179.

50. Pfarr, C.M.; Coue, M.; Grissom, P.M.; Hays, T.S.; Porter, M.E.; McIntosh, J.R. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature* 1990, 345, 263-265.

51. Steuer, E.R.; Wordeman, L.; Schroer, T.A.; Sheetz, M.P. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature* 1990, 345, 266-268.

52. Kapoor, T.M.; Lampson, M.A.; Hergert, P.; Cameron, L.; Cinini, D.; Salmon, E.D.; McEwen, B.F.; Khodjakov, A. Chromosomes can congress to the metaphase plate before biorientation. *Science (New York, N.Y)* 2006, 311, 388-391.

53. Wood, K.W.; Sakowicz, R.; Goldstein, L.S.; Cleveland, D.W. Cenpe is a plus end-directed kinesin motor required for metaphase chromosome alignment. *Dev Cell* 2004, 6, 253-268.

54. Mann, B.J.; Wadsworth, P. Kinesin-5 regulation and function in mitosis. *Trends in cell biology* 2019, 29, 66-79.

55. Bakhoun, S.F.; Thompson, S.L.; Manning, A.L.; Compton, D.A. Genome stability is ensured by temporal control of kinetochore-microtubule dynamics. *Nat Cell Biol* 2009, 11, 27-35.

56. Kline-Smith, S.L.; Khodjakov, A.; Hergert, P.; Walczak, C.E. Depletion of centromeric mcak leads to chromosome congression and segregation defects due to improper kinetochore attachments. *Molecular biology of the cell* 2004, 15, 1146-1159.

57. Lan, W.; Zhang, X.; Kline-Smith, S.L.; Rosasco, S.E.; Barrett-Wilt, G.A.; Shabanowitz, J.; Hunt, D.F.; Walczak, C.E.; Stukenberg, P.T. Aurora b phosphorylates centromeric mcak and regulates its localization and microtubule depolymerization activity. *Curr Biol* 2004, 14, 273-286.

58. Andrews, P.D.; Ovechkina, Y.; Morris, N.; Wagenbach, M.; Duncan, K.; Wordeman, L.; Swedlow, J.R. Aurora b regulates mcak at the mitotic centromere. *Developmental cell* 2004, 6, 253-268.

59. Domnitz, S.B.; Wagenbach, M.; Decarreau, J.; Wordeman, L. Mcak activity at microtubule tips regulates spindle microtubule length to promote robust kinetochore attachment. *The Journal of cell biology* 2012, 197, 231-237.

60. Reed, N.A.; Cai, D.; Blasius, T.L.; Jih, G.T.; Meyhofer, E.; Gaertig, J.; Verhey, K.J. Microtubule acetylation promotes kinesin-1 binding and transport. *Curr Biol* 2006, 16, 2166-2172.

61. Konishi, Y.; Setou, M. Tubulin tyrosination navigates the kinesin-1 motor domain to axons. *Nature Neuroscience* 2009, 12, 559-567.

62. Pfarr, C.M.; Coue, M.; Grissom, P.M.; Hays, T.S.; Porter, M.E.; McIntosh, J.R. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature* 1990, 345, 263-265.

63. McKenney, R.J.; Huynh, W.; Tanenbaum, M.E.; Bhabha, G.; Vale, R.D. Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. *Science* 2014, 345, 337-341.
64. McKenney, R.J.; Huynh, W.; Vale, R.D.; Sirajuddin, M. Tyrosination of alpha-tubulin controls the initiation of processive dynein-dynactin motility. *Embo J* **2016**, *35*, 1175-1185.

65. Nirschl, J.J.; Magiera, M.M.; Lazarus, J.E.; Janke, C.; Holzbaur, E.L. Alpha-tubulin tyrosination and clip-170 phosphorylation regulate the initiation of dynein-driven transport in neurons. *Cell Rep* **2016**, *14*, 2637-2652.

66. Barisic, M.; Maiato, H. The tubulin code: A navigation system for chromosomes during mitosis. *Trends Cell Biol* **2016**, *26*, 766-775.

67. Barisic, M.; Aguiar, P.; Geley, S.; Maiato, H. Kinetochore motors drive congression of peripheral polar chromosomes by overcoming random arm-ejection forces. *Nat Cell Biol* **2014**, *16*, 1249-1256.

68. Chmatal, L.; Yang, K.; Schultz, R.M.; Lampson, M.A. Spatial regulation of kinetochore microtubule attachments by destabilization at spindle poles in meiosis i. *Curr Biol* **2015**, *25*, 1835-1841.

69. Ye, A.A.; Deretic, J.; Hole, C.M.; Hinman, A.W.; Cimini, D.; Welburn, J.P.; Maresca, T.J. Aurora a kinase contributes to a pole-based error correction pathway. *Mol Biol Cell* **2015**, *26*, 70.

70. Kim, Y.; Holland, A.J.; Lan, W.; Cleveland, D.W. Aurora kinases and protein phosphatase 1 mediate chromosome congression through regulation of cemp-e. *Cell* **2010**, *142*, 444-455.

71. Pereira, A.; Sousa, M.; Almeida, A.C.; Ferreira, L.T.; Costa, A.R.; Novais-Cruz, M.; Ferras, C.; Sousa, M.M.; Sampaio, P.; Belsley, M., *et al*. Coherent-hybrid sted: High contrast sub-diffraction imaging using a bi-vortex depletion beam. *Optics express* **2019**, *27*, 8092-8111.

72. Steblyanko, Y., Rajendraprasad, G., Osswald, M., Eibes, S., Jacome, A., Geley, S., Pereira, A.J., Maiato, H. and Barisic, M. Microtubule poleward flux in human cells is driven by the coordinated action of four kinesins. *The EMBO journal* **2020**, e105432.

73. Bakhoun, S.F.; Genovese, G.; Compton, D.A. Deviant kinetochore microtubule dynamics underlie chromosomal instability. *Curr Biol* **2009**, *19*, 1937-1942.

74. Bakhoun, S.F.; Ngo, B.; Laughney, A.M.; Cavallo, J.A.; Murphy, C.J.; Ly, P.; Shah, P.; Sriram, R.K.; Watkins, T.B.K.; Taunk, N.K., *et al*. Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* **2018**, *553*, 467-472.

75. Peris, L.; Wagenbach, M.; Lafanechere, L.; Brocard, J.; Moore, A.T.; Kozielski, F.; Job, D.; Wordeman, L.; Andrieux, A. Motor-dependent microtubule disassembly driven by tubulin tyrosination. *The Journal of cell biology* **2009**, *185*, 1159-1166.

76. Sirajuddin, M.; Rice, L.M.; Vale, R.D. Regulation of microtubule motors by tubulin isotypes and post-translational modifications. *Nat Cell Biol* **2014**, *16*, 335-344.

77. Silkworth, W.T.; Nardi, I.K.; Paul, R.; Mogilner, A.; Cimini, D. Timing of centrosome separation is important for accurate chromosome segregation. *Mol Biol Cell* **2012**, *23*, 401-411.

78. Nunes, V.; Dantas, M.; Castro, D.; Vitiello, E.; Wang, I.; Carpi, N.; Balland, M.; Piel, M.; Aguiar, P.; Maiato, H., *et al*. Centrosome-nuclear axis repositioning drives the assembly of a bipolar spindle scaffold to ensure mitotic fidelity. *Mol Biol Cell* **2020**, mbeE20010047.

79. Raaijmakers, J.A.; van Heesbeen, R.G.H.P.; Meaders, J.L.; Geers, E.F.; Fernandez-Garcia, B.; Medema, R.H.; Tanenbaum, M.E. Nuclear envelope-associated dynein drives prophase centrosome separation and enables eg5-independent bipolar spindle formation. *Embo J* **2012**, *31*, 4179-4190.

80. Kahn, O.I.; Sharma, V.; Gonzalez-Billault, C.; Baas, P.W. Effects of kinesin-5 inhibition on dendritic architecture and microtubule organization. *Mol Biol Cell* **2015**, *26*, 66-77.

81. Siller, K.H.; Doe, C.Q. Spindle orientation during asymmetric cell division. *Nat Cell Biol* **2009**, *11*, 365-374.
82. Kotak, S.; Busso, C.; Gonczy, P. Cortical dynein is critical for proper spindle positioning in human cells. *The Journal of cell biology* 2012, 199, 97-110.

83. Nguyen-Ngoc, T.; Afshar, K.; Gonczy, P. Coupling of cortical dynein and g alpha proteins mediates spindle positioning in caenorhabditis elegans. *Nature cell biology* 2007, 9, 1294-1302.

84. Barbosa, D.J.; Duro, J.; Prevo, B.; Cheerambathur, D.K.; Carvalho, A.X.; Gassmann, R. Dynactin binding to tyrosinated microtubules promotes centrosome centration in c. Elegans by enhancing dynein-mediated organelle transport. *PLoS Genet* 2017, 13, e1006941.

85. Bobinnec, Y.; Khodjakov, A.; Mir, L.M.; Rieder, C.L.; Edde, B.; Bornens, M. Centriole disassembly in vivo and its effect on centrosome structure and function in vertebrate cells. *Journal of Cell Biology* 1998, 143, 1575-1589.

86. Abal, M.; Keryer, G.; Bornens, M. Centrioles resist forces applied on centrosomes during g2/m transition. *Biology of the Cell* 2005, 97, 425-434.

87. Mahecic, D.; Gambarotto, D.; Douglass, K.M.; Fortun, D.; Banterle, N.; Ibrahim, K.A.; Le Guennec, M.; Gonczy, P.; Hamel, V.; Guichard, P., et al. Homogeneous multifocal excitation for high-throughput super-resolution imaging. *Nat Methods* 2020, 17, 726-733.

88. LaCroix, B.; van Dijk, J.; Gold, N.D.; Guizetti, J.; Aldrian-Herrada, G.; Rogowski, K.; Gerlich, D.W.; Janke, C. Tubulin polyglutamylation stimulates spastin-mediated microtubule severing. *Journal of Cell Biology* 2010, 189, 945-954.

89. Sharma, N.; Bryant, J.; Wloga, D.; Donaldson, R.; Davis, R.C.; Jerka-Dziadosz, M.; Gaertig, J. Katanin regulates dynamics of microtubules and biogenesis of motile cilia. *Journal of Cell Biology* 2007, 178, 1065-1079.

90. Shin, S.C.; Im, S.K.; Jang, E.H.; Hur, E.M.; Kim, E.E. Structural and molecular basis for katanin-mediated severing of glutamylated microtubules. *Cell Reports* 2019, 26, 1357-+.

91. Valenstein, M.L.; Roll-Mecak, A. Graded control of microtubule severing by tubulin glutamylation. *Cell* 2016, 164, 911-921.

92. Jiang, K.; Rezabkova, L.; Hua, S.S.; Liu, Q.Y.; Capitani, G.; Altelaar, A.F.M.; Heck, A.J.R.; Kammerer, R.A.; Steinmetz, M.O.; Akhmanova, A. Microtubule minus-end regulation at spindle poles by an aspm-katanin complex (vol 19, pg 480, 2017). *Nature Cell Biology* 2017, 19, 873-873.

93. McNally, K.; Audhya, A.; Oegema, K.; McNally, F.J. Katanin controls mitotic and meiotic spindle length. *Journal of Cell Biology* 2006, 175, 881-891.

94. Zhang, D.; Rogers, G.C.; Buster, D.W.; Sharp, D.J. Three microtubule severing enzymes contribute to the "pacman-flux" machinery that moves chromosomes. *Journal of Cell Biology* 2007, 177, 231-242.

95. Connell, J.W.; Lindon, C.; Luzio, J.P.; Reid, E. Spastin couples microtubule severing to membrane traffic in completion of cytokinesis and secretion. *Traffic* 2009, 10, 42-56.

96. Guizetti, J.; Schermelleh, L.; Mantler, J.; Maar, S.; Poser, I.; Leonhardt, H.; Muller-Reichert, T.; Gerlich, D.W. Cortical constriction during abscission involves helices of escrt-iii-dependent filaments. *Science* 2011, 331, 1616-1620.

97. Matsuo, M.; Shimodaira, T.; Kasama, T.; Hata, Y.; Echigo, A.; Okabe, M.; Arai, K.; Makino, Y.; Niwa, S.I.; Saya, H., et al. Katanin p60 contributes to microtubule instability around the midbody and facilitates cytokinesis in rat cells. *Plos One* 2013, 8.

98. Thazhath, R.; Liu, C.B.; Gaertig, J. Polyglycylation domain of beta-tubulin maintains axonemal architecture and affects cytokinesis in tetrahymena. *Nature Cell Biology* 2002, 4, 256-259.
99. Boggs, A.E.; Vitolo, M.I.; Whipple, R.A.; Charpentier, M.S.; Goloubeva, O.G.; Ioffe, O.B.; Tuttle, K.C.; Slovic, J.; Lu, Y.; Mills, G.B., et al. Alpha-tubulin acetylation elevated in metastatic and basal-like breast cancer cells promotes microtentacle formation, adhesion, and invasive migration. Cancer Res 2015, 75, 203-215.

100. Mialhe, A.; Lafanechere, L.; Treilleux, I.; Peloux, N.; Dumontet, C.; Bremond, A.; Panh, M.H.; Payan, R.; Wehland, J.; Margolis, R.L., et al. Tubulin detyrosination is a frequent occurrence in breast cancers of poor prognosis. Cancer Res 2001, 61, 5024-5027.

101. Kato, C.; Miyazaki, K.; Nakamura, A.; Ohira, M.; Nakamura, Y.; Ozaki, T.; Imai, T.; Nakagawara, A. Low expression of human tubulin tyrosine ligase and suppressed tubulin tyrosination/detyrosination cycle are associated with impaired neuronal differentiation in neuroblastomas with poor prognosis. International Journal of Cancer 2004, 112, 365-375.

102. Soucek, K.; Kamai, A.; Phung, A.D.; Kubala, L.; Bulinski, J.C.; Harper, R.W.; Eiserich, J.P. Normal and prostate cancer cells display distinct molecular profiles of alpha-tubulin posttranslational modifications. Prostate 2006, 66, 954-965.

103. Whipple, R.A.; Matrone, M.A.; Cho, E.H.; Balzer, E.M.; Vitolo, M.I.; Yoon, J.R.; Ioffe, O.B.; Tuttle, K.C.; Yang, J.; Martin, S.S. Epithelial-to-mesenchymal transition promotes tubulin detyrosination and microtentacles that enhance endothelial engagement. Cancer Res 2010, 70, 8127-8137.

104. Xue, X.; Gao, W.; Sun, B.; Xu, Y.; Han, B.; Wang, F.; Zhang, Y.; Sun, J.; Wei, J.; Lu, Z., et al. Vasohibin 2 is transcriptionally activated and promotes angiogenesis in hepatocellular carcinoma. Oncogene 2013, 32, 1724-1734.

105. Li, D.W.; Sun, X.D.; Zhang, L.L.; Yan, B.; Xie, S.B.; Liu, R.M.; Liu, M.; Zhou, J. Histone deacetylase 6 and cytoplasmic linker protein 170 function together to regulate the motility of pancreatic cancer cells. Protein Cell 2014, 5, 214-223.

106. Wang, Z.; Hu, P.; Tang, F.; Lian, H.; Chen, X.; Zhang, Y.; He, X.; Liu, W.; Xie, C. Hdac6 promotes cell proliferation and confers resistance to temozolomide in glioblastoma. Cancer Lett 2016, 379, 134-142.

107. Gradilone, S.A.; Radtke, B.N.; Bogert, P.S.; Huang, B.Q.; Gajdos, G.B.; LaRusso, N.F. Hdac6 inhibition restores ciliary expression and decreases tumor growth. Cancer Res 2013, 73, 2259-2270.

108. Kashiwaya, K.; Nakagawa, H.; Hosokawa, M.; Mochizuki, Y.; Ueda, K.; Piao, L.; Chung, S.; Hamamoto, R.; Eguchi, H.; Ohigashi, H., et al. Involvement of the tubulin tyrosine ligase-like family member 4 polyglutamylase in pelp1 polyglutamylation and chromatin remodeling in pancreatic cancer cells. Cancer Res 2010, 70, 4024-4033.

109. Rocha, C.; Papon, L.; Cacheux, W.; Marques Sousa, P.; Lancano, V.; Tort, O.; Giordano, T.; Vacher, S.; Lemmers, B.; Mariani, P., et al. Tubulin glycolases are required for primary cilia, control of cell proliferation and tumor development in colon. The EMBO journal 2014, 33, 2247-2260.

110. McCarroll, J.A.; Sharbeen, G.; Liu, J.; Youkhana, J.; Goldstein, D.; McCarthy, N.; Limbri, L.F.; Dischl, D.; Ceyhan, G.O.; Erkan, M., et al. Betaiii-tubulin: A novel mediator of chemoresistance and metastases in pancreatic cancer. Oncotarget 2015, 6, 2235-2249.

111. Lee, K.M.; Cao, D.; Itami, A.; Pour, P.M.; Hruban, R.H.; Maitra, A.; Ouellette, M.M. Class iii beta-tubulin, a marker of resistance to paclitaxel, is overexpressed in pancreatic ductal adenocarcinoma and intraepithelial neoplasia. Histopathology 2007, 51, 539-546.

112. Kanojia, D.; Morshed, R.A.; Zhang, L.; Miska, J.M.; Qiao, J.; Kim, J.W.; Pytel, P.; Balysnikova, I.V.; Lesniak, M.S.; Ahmed, A.U. Betaiiii-tubulin regulates breast cancer metastases to the brain. Mol Cancer Ther 2015, 14, 1152-1161.
113. McCarroll, J.A.; Gan, P.P.; Liu, M.; Kavallaris, M. Beta ii-tubulin is a multifunctional protein involved in drug sensitivity and tumorigenesis in non-small cell lung cancer. *Cancer Res* 2010, 70, 4995-5003.

114. Ferrandina, G.; Zannoni, G.F.; Martinelli, E.; Paglia, A.; Gallotta, V.; Mozzetti, S.; Scambia, G.; Ferlini, C. Class iii beta-tubulin overexpression is a marker of poor clinical outcome in advanced ovarian cancer patients. *Clin Cancer Res* 2006, 12, 2774-2779.

115. Ruksha, K.; Mezheyevski, A.; Nerovnya, A.; Bich, T.; Tur, G.; Gorgun, J.; Luuquena, R.; Portyanko, A. Over-expression of beta ii-tubulin and especially its localization in cell nuclei correlates with poorer outcomes in colorectal cancer. *Cells* 2019, 8.

116. Parker, A.L.; Teo, W.S.; McCarroll, J.A.; Kavallaris, M. An emerging role for tubulin isotypes in modulating cancer biology and chemotherapy resistance. *International journal of molecular sciences* 2017, 18.

117. Panda, D.; Miller, H.P.; Banerjee, A.; Luuquena, R.F.; Wilson, L. Microtubule dynamics in vitro are regulated by the tubulin isotype composition. *Proceedings of the National Academy of Sciences of the United States of America* 1994, 91, 11358-11362.

118. Pamula, M.C.; Ti, S.C.; Kapoor, T.M. The structured core of human beta tubulin confers isotype-specific polymerization properties. *The Journal of cell biology* 2016, 213, 425-433.

119. Ti, S.C.; Alushin, G.M.; Kapoor, T.M. Human beta-tubulin isotypes can regulate microtubule protofilament number and stability. *Developmental cell* 2018, 47, 175-190 e175.

120. van Dijk, J.; Miro, J.; Strub, J.M.; Lacroix, B.; van Dorsselaer, A.; Edde, B.; Janke, C. Polyglutamylation is a post-translational modification with a broad range of substrates. *The Journal of biological chemistry* 2008, 283, 3915-3922.

121. Regnard, C.; Desbryueres, E.; Huet, J.C.; Beauvallet, C.; Pernollet, J.C.; Edde, B. Polyglutamylation of nucleosome assembly proteins. *The Journal of biological chemistry* 2000, 275, 15969-15976.

122. Aguilar, A.; Becker, L.; Tedeschi, T.; Heller, S.; Iomini, C.; Nachury, M.V. Alpha-tubulin k40 acetylation is required for contact inhibition of proliferation and cell-substrate adhesion. *Mol Biol Cell* 2014, 25, 1854-1866.

123. Lee, Y.S.; Lim, K.H.; Guo, X.; Kawaguchi, Y.; Gao, Y.; Barrientos, T.; Ordentlich, P.; Wang, X.F.; Counter, C.M.; Yao, T.P. The cytoplasmic deacetylase hdac6 is required for efficient oncogenic tumorigenesis. *Cancer Res* 2008, 68, 7561-7569.

124. Putcha, P.; Yu, J.; Rodriguez-Barrueco, R.; Saucedo-Cuevas, L.; Villafrasa, P.; Murga-Penas, E.; Quayle, S.N.; Yang, M.; Castro, V.; Llobet-Navas, D., et al. Hdac6 activity is a non-oncogene addiction hub for inflammatory breast cancers. *Breast Cancer Res* 2015, 17, 149.

125. Woan, K.V.; Lienlaf, M.; Perez-Villaruel, P.; Lee, C.; Cheng, F.; Knox, T.; Woods, D.M.; Barrios, K.; Powers, J.; Sahakian, E., et al. Targeting histone deacetylase 6 mediates a dual anti-melanoma effect: Enhanced antitumor immunity and impaired cell proliferation. *Mol Oncol* 2015, 9, 1447-1457.

126. Zhang, X.; Yuan, Z.; Zhang, Y.; Yong, S.; Salas-Burgos, A.; Koomen, J.; Olashaw, N.; Parsons, J.T.; Yang, X.J.; Dent, S.R., et al. Hdac6 modulates cell motility by altering the acetylation level of cortactin. *Mol Cell* 2007, 27, 197-213.

127. Lafanecere, L.; Couratay-Cahen, C.; Kawakami, T.; Jacrot, M.; Rudiger, M.; Wehland, J.; Job, D.; Margolis, R.L. Suppression of tubulin tyrosine ligase during tumor growth. *Journal of cell science* 1998, 111 ( Pt 2), 171-181.

128. Sato, Y. The vasohibin family: A novel family for angiogenesis regulation. *J Biochem* 2013, 153, 5-11.
129. Kimura, H.; Miyashita, H.; Suzuki, Y.; Kobayashi, M.; Watanabe, K.; Sonoda, H.; Ohta, H.; Fujiwara, T.; Shimosegawa, T.; Sato, Y. Distinctive localization and opposed roles of vasohibin-1 and vasohibin-2 in the regulation of angiogenesis. *Blood* 2009, 113, 4810-4818.

130. Heishi, T.; Hosaka, T.; Suzuki, Y.; Miyashita, H.; Oike, Y.; Takahashi, T.; Nakamura, T.; Arioka, S.; Mitsuda, Y.; Takakura, T., et al. Endogenous angiogenesis inhibitor vasohibin1 exhibits broad-spectrum antilymphangiogenic activity and suppresses lymph node metastasis. *Am J Pathol* 2010, 176, 1950-1958.

131. Hosaka, T.; Kimura, H.; Heishi, T.; Suzuki, Y.; Miyashita, H.; Ohta, H.; Sonoda, H.; Moriya, T.; Suzuki, S.; Kondo, T., et al. Vasohibin-1 expression in endothelium of tumor blood vessels regulates angiogenesis. *Am J Pathol* 2009, 175, 430-439.

132. Kitahara, S.; Suzuki, Y.; Morishima, M.; Yoshii, A.; Kikuta, S.; Shimizu, K.; Morikawa, S.; Sato, Y.; Ezaki, T. Vasohibin-2 modulates tumor onset in the gastrointestinal tract by normalizing tumor angiogenesis. *Mol Cancer* 2014, 13, 99.

133. Koyanagi, T.; Suzuki, Y.; Saga, Y.; Machida, S.; Takei, Y.; Fujiwara, H.; Suzuki, M.; Sato, Y. In vivo delivery of sirna targeting vasohibin-2 decreases tumor angiogenesis and suppresses tumor growth in ovarian cancer. *Cancer Sci* 2013, 104, 1705-1710.

134. Takahashi, Y.; Koyanagi, T.; Suzuki, Y.; Saga, Y.; Kanomata, N.; Moriya, T.; Suzuki, M.; Sato, Y. Vasohibin-2 expressed in human serous ovarian adenocarcinoma accelerates tumor growth by promoting angiogenesis. *Mol Cancer Res* 2012, 10, 1135-1146.

135. Pagnamenta, A.T.; Heemeryck, P.; Martin, H.C.; Bosc, C.; Peris, L.; Uszynski, I.; Gory-Faure, S.; Couly, S.; Deshpande, C.; Siddiqui, A., et al. Defective tubulin detyrosination causes structural brain abnormalities with cognitive deficiency in humans and mice. *Hum Mol Genet* 2019, 28, 3391-3405.

136. Gu, S.; Liu, Y.; Zhu, B.; Ding, K.; Yao, T.P.; Chen, F.; Zhan, L.; Xu, P.; Ehrlich, M.; Liang, T., et al. Loss of alpha-tubulin acetylation is associated with tgf-beta-induced epithelial-mesenchymal transition. *J Biol Chem* 2016, 291, 5396-5405.

137. Bance, B.; Seetharaman, S.; Leduc, C.; Boeda, B.; Etienne-Manneville, S. Microtubule acetylation but not detyrosination promotes focal adhesion dynamics and astrocyte migration. *Journal of cell science* 2019, 132.

138. Haggarty, S.J.; Koeller, K.M.; Wong, J.C.; Grozinger, C.M.; Schreiber, S.L. Domain-selective small-molecule inhibitor of histone deacetylase 6 (hdac6)-mediated tubulin deacetylation. *Proc Natl Acad Sci U S A* 2003, 100, 4389-4394.

139. Castro-Castro, A.; Janke, C.; Montagnac, G.; Paul-Gilloteaux, P.; Chavrier, P. Atat1/mec-17 acetyltransferase and hdac6 deacetylase control a balance of acetylation of alpha-tubulin and cortactin and regulate mtl-mmp trafficking and breast tumor cell invasion. *Eur J Cell Biol* 2012, 91, 950-960.

140. Montagnac, G.; Meas-Yedid, V.; Irontelle, M.; Castro-Castro, A.; Franco, M.; Shida, T.; Nachury, M.V.; Benmerah, A.; Olivo-Marin, J.C.; Chavrier, P. Alphatat1 catalyses microtubule acetylation at clathrin-coated pits. *Nature* 2013, 502, 567-570.

141. Tran, A.D.; Marmo, T.P.; Salam, A.A.; Che, S.; Finkelstein, E.; Kabarriti, R.; Xenias, H.S.; Mazitschek, R.; Hubbert, C.; Kawaguchi, Y., et al. Hdac6 deacetylation of tubulin modulates dynamics of cellular adhesions. *J Cell Sci* 2007, 120, 1469-1479.

142. Rey, M.; Irontelle, M.; Wåharte, F.; Lizarra, F.; Chavrier, P. Hdac6 is required for invadopodia activity and invasion by breast tumor cells. *Eur J Cell Biol* 2011, 90, 128-135.
143. Whipple, R.A.; Vitolo, M.I.; Boggs, A.E.; Charpentier, M.S.; Thompson, K.; Martin, S.S. Parthenolide and costunolide reduce microtentacles and tumor cell attachment by selectively targeting detyrosinated tubulin independent from nf-kappab inhibition. *Breast Cancer Res* 2013, 15, R83.

144. Fonrose, X.; Ausseil, F.; Soleilhac, E.; Masson, V.; David, B.; Pouny, I.; Cintrat, J.C.; Rousseau, B.; Barette, C.; Massiot, G., *et al.* Parthenolide inhibits tubulin carboxypeptidase activity. *Cancer Res* 2007, 67, 3371-3378.

145. Clemente-Ruiz, M.; Muzzopappa, M.; Milan, M. Tumor suppressor roles of cenp-e and nsl1 in drosophila epithelial tissues. *Cell Cycle* 2014, 13, 1450-1455.

146. Silk, A.D.; Zasadil, L.M.; Holland, A.J.; Vitre, B.; Cleveland, D.W.; Weaver, B.A. Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. *Proceedings of the National Academy of Sciences of the United States of America* 2013, 110, E4134-4141.

147. Weaver, B.A.; Silk, A.D.; Montagna, C.; Verdier-Pinard, P.; Cleveland, D.W. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* 2007, 11, 25-36.

148. Zasadil, L.M.; Britigan, E.M.; Ryan, S.D.; Kaur, C.; Guckenberger, D.J.; Beebe, D.J.; Moser, A.R.; Weaver, B.A. High rates of chromosome missegregation suppress tumor progression but do not inhibit tumor initiation. *Mol Biol Cell* 2016, 27, 1981-1989.