Focus Article

Microtubule-based force generation

Ian A. Kent and Tanmay P. Lele*

Microtubules are vital to many important cell processes, such as cell division, transport of cellular cargo, organelle positioning, and cell migration. Owing to their diverse functions, understanding microtubule function is an important part of cell biological research that can help in combating various diseases. For example, microtubules are an important target of chemotherapeutic drugs such as paclitaxel because of their pivotal role in cell division. Many functions of microtubules relate to the generation of mechanical forces. These forces are generally either a direct result of microtubule polymerization/depolymerization or generated by motor proteins that move processively along microtubules. In this review, we summarize recent efforts to quantify and model force generation by microtubules in the context of microtubule function. © 2016 Wiley Periodicals, Inc.

How to cite this article:
WIREs Nanomed Nanobiotechnol 2016. doi: 10.1002/wnan.1428

INTRODUCTION

Microtubules are a vital component of the cytoskeleton present in all eukaryotic cells. They are filamentous, tubular biopolymers assembled from αβ-tubulin heterodimers arranged into 13 protofilaments.[1] In mammals, microtubule networks in undifferentiated and many differentiated cell types take on an archetypal astral arrangement centered at the centrosome, a small organelle that nucleates microtubules primarily via the γ-tubulin ring complex. Microtubules can also be assembled *in vitro*; these can have varying numbers of protofilaments when not seeded from a nucleator such as a centrosome.[1,2]

Microtubules undergo constant cycles of polymerization and depolymerization by a GTP-dependent process known as dynamic instability.[1,3] Microtubules self-assemble in the presence of GTP and require GTP to be bound to β-tubulin for individual dimers to be added. During polymerization *in vivo*, new GTP-αβ-tubulin dimers are added to the plus end of the microtubule from the cytoplasmic pool. These new tubulin subunits form a GTP-tubulin 'cap' at the plus end that stabilizes the microtubule.[1,4] Away from the GTP-tubulin cap, the GTP bound to β-tubulin is hydrolyzed, such that the rest of the microtubule is mostly made up of GDP-tubulin subunits. If the GTP cap is lost due to hydrolysis, the microtubule enters catastrophe, a rapid state of depolymerization from the plus end. In recent years, evidence has been found to support the presence of unhydrolyzed 'islands' of GTP-tubulin away from the plus end, which likely aid in rescue of polymerization following catastrophe.[5,6] Microtubules have built-in structural polarity with different polymerization kinetics at their two ends. The microtubule plus end polymerizes more quickly *in vitro* than the minus end. *In vivo*, free minus ends either do not polymerize or polymerize very little owing to the presence of capping proteins such as calmodulin-regulated spectrin-associated proteins in vertebrates and Patronin in invertebrates.[7–9] Meanwhile, plus ends are regulated by a large number of plus tip tracking proteins known as the +TIPs (see section on *Polymerization Forces*).

The addition of new GTP-tubulin dimers to and the removal of GDP-tubulin dimers from the plus end are both energetically favored, with a free energy change ΔG of 5–10 k_BT.[1] Both polymerization and depolymerization can provide energy to perform mechanical work in the cell. Thus, a microtubule growing against a barrier can exert a pushing force, while a depolymerizing microtubule

*Correspondence to: tlele@che.ufl.edu

Department of Chemical Engineering, University of Florida, Gainesville, FL, USA

Conflict of interest: The authors have declared no conflicts of interest for this article.

whose tip continues to be attached to another structure can pull, as occurs with kinetochore microtubules.[10]

Microtubules are relatively stiff, with a flexural rigidity of ~22 pN μm^2 and a Young's modulus on the order of 1 GPa.[11] This corresponds to a persistence length under purely thermal forces of the order of millimeters; by comparison, double-stranded DNA is much softer with a persistence length of tens of nanometers.[11–13] During mitosis, microtubules are the primary component of the mitotic spindle, which aligns and separates chromosomes between the two resultant daughter cells. In migratory adherent cells, crosslinking between microtubules and other cytoskeletal elements may help to stabilize the leading edge.[14] Processive molecular motor proteins associated with microtubules are responsible for transport of various cargos throughout the cell and act to spatially organize the microtubule network. Many of the diverse functions of microtubules are directly tied to generation of force via microtubule polymerization, microtubule depolymerization, or molecular motors. In this review, we discuss microtubule-based force generation and its relevance in targeting microtubules for cancer treatment.

MICROTUBULE FORCES

Polymerization Forces

In *in vitro* experiments, polymerizing microtubules have been shown to exert force upon boundaries in a force- and concentration-dependent manner, consistent with the Brownian ratchet mechanism of force generation (Figure 1(a)). In this model, thermal energy causes fluctuations in the position of the barrier, allowing subunits to enter in between the tip and the barrier.[15,16] When a force is applied in opposition to a growing microtubule tip, the average amount of space in between the microtubule tip and the barrier is reduced. Therefore, it becomes less probable at any given moment for new tubulin dimers to diffuse into the space in between the microtubule and the boundary. Growth can be reduced and eventually stopped as the force is increased. At even higher forces, catastrophe can be induced as well.[17] The growth velocity of microtubules decays exponentially as an opposing force is applied to the tip; however, polymerization still occurs up to a force of about 5 pN.[18] Multiple models have been proposed to explain the force–velocity relationship of a growing microtubule against a boundary.[19]

Pushing forces by polymerization of microtubules have been implicated in subcellular processes such as organelle positioning. For example, in *Schizosaccharomyces pombe* (fission yeast), the nucleus has been proposed to be centered by a pushing mechanism in which microtubule bundles originating at the nucleus polymerize against the cell cortex. This polymerization of microtubule bundles is accompanied by nuclear motion away from the cortex and deformation of the nuclear envelope.[20,21] In a recent study investigating the mitotic spindle in one- and two-cell *Caenorhabditis elegans* embryos, forces on the order of tens of picoNewtons were sufficient to displace a centrosome away from the original axis of the mitotic spindle. The stiffness of the spindle to displacement was dependent on the number of astral microtubules, the phase of mitosis (anaphase spindles were stiffer than metaphase), and cell size (spindles were stiffer in smaller cells vs larger cells).[22] These differences in stiffness correlated with the number of astral microtubules reaching the cell cortex, an indication that microtubule polymerization forces could play an important role in the centering mechanism of the *C. elegans* spindle.[22] In larger eukaryotic cells, it has been argued that pushing forces are insufficient to center cellular structures such as the centrosome owing to the much larger frictional resistance on microtubules in these cells as well as the lower amount of force necessary to buckle a microtubule the longer it grows.[19,23]

Microtubules are able to deform structures in the cell as they polymerize into them. Microtubule polymerization forces have been observed to deform the nucleus in mammalian cells just before the nuclear envelope breakdown (NEB) that occurs before mitosis, and this interaction between microtubules and the nucleus may trigger the initiation of NEB.[24] Similar nuclear indentations have been observed upon regrowth of microtubules from the centrosome after microtubule depolymerization with nocodazole, with heterochromatin enriched near these indentations.[25] Microtubules have also been observed to deform and even break through the plasma membrane to produce filopodia-like protrusions in conditions where the cell is damaged (transection of an axon).[26]

In vivo, the microtubule plus end is associated with a cohort of plus-end-tracking proteins (+TIPs) that affect polymerization rate and therefore likely affect the pushing forces that polymerizing microtubules are able to generate.[9] The end-binding (EB) proteins, a type of +TIP protein, form a tip-tracking complex. This complex increases the polymerization rate by inducing a structural change near

the plus end and recruiting other +TIPs. The XMAP215/Dis1 and CLASP families of +TIPs recruit tubulin dimer subunits to the plus end, increasing the rate of microtubule polymerization.[27] CLASPs have been shown to suppress catastrophe as well.[27,28] Cap-Gly domain proteins such as CLIP-170 have been shown to promote microtubule rescue.[29–31] The largest subunit of dynactin, p150^{Glued}, also a Cap-Gly domain protein, localizes and regulates microtubule dynamics differently depending on how it is spliced in different tissues.[32] The isoform of p150^{Glued} that is expressed in neurons acts to suppress catastrophe, while the isoform present in epithelial cells does not seem to affect rate of catastrophe.[33] Further research is needed in understanding the effects these different EB proteins have on polymerization pushing forces.

In addition, various kinesins have been shown to affect microtubule dynamics. Members of the kinesin-8 family process toward the plus end, where they accumulate and cooperatively destabilize the GTP cap, inducing catastrophe. Members of the kinesin-4 family decrease overall turnover at the microtubule plus end, stabilizing microtubules at a specific length in contexts such as the mitotic spindle and cell cortex. The kinesin-13 family member mitotic centromere-associated kinesin (MCAK) is able to diffuse one-dimensionally along microtubules and induce depolymerization at either the plus or minus end.[34] *In vitro* experiments have demonstrated its ability to generate a pulling force by tracking the depolymerizing tip.[34] Overexpression of MCAK causes spindle defects and contributes to paclitaxel resistance in cancer.[35] As such, MCAK has received attention as a possible drug target in cancer.[36] A review of kinesins that regulate microtubule dynamics is available from Su et al.[37]

Depolymerization Forces

Just as microtubule polymerization can generate pushing forces, depolymerization can also be harnessed to generate pulling forces when a link is

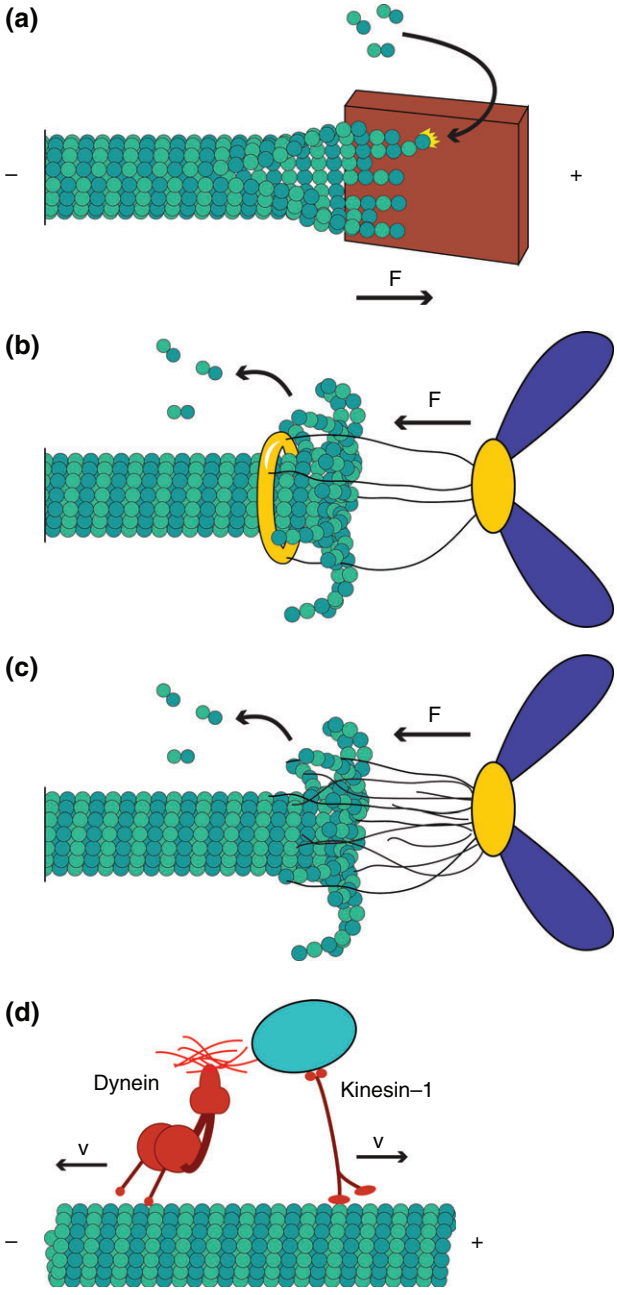

FIGURE 1 | Legend on next column.

FIGURE 1 | (a) Force production by polymerization of a microtubule against a boundary. Insertion of new αβ-tubulin subunits at the plus end exerts a pushing force on the boundary (brown). If the boundary resists movement, this force can cause flux of the microtubule toward the minus end. (b) One possible mechanism for the conformational wave model of microtubule depolymerization exerting a pulling force to separate chromosomes (purple) during mitosis. The kinetochore (yellow oval) is attached to a ring-shaped complex (yellow annulus) via protein tethers (black lines). As the microtubule depolymerizes, the curling of tubulin protofilaments performs a 'power stroke' on the ring-shaped complex, and a pulling force is exerted on the kinetochore. (c) An alternative mechanism to (b) known as the biased diffusion model. In this model, kinetochore proteins (black lines) interact transiently with a microtubule. As the microtubule depolymerizes, motion of the kinetochore toward the microtubule tip is energetically favored, because when the kinetochore is closer to the microtubule tip, more kinetochore proteins are able to bind to the microtubule lattice. (d) Forces generated by microtubule motors. Cytoplasmic dynein and kinesin-1 are shown. These are, respectively, the primary minus end-directed and plus end-directed motors that transport cargo within the cell. When a motor is anchored at its cargo end, for example to other elements of the cytoskeleton (red lines), the motor exerts a force on the microtubule in the opposite direction of its processivity.

maintained between an object and the depolymerizing microtubule tip. The main cellular process in which microtubule depolymerization forces play a role is the separation of chromosomes during mitosis. During mitosis, the centromeres of each chromosome recruit a large kinetochore complex, through which the condensed chromosomes are attached to the microtubules of the mitotic spindle.[38] Although the kinetochore complex contains upward of 100 different proteins, certain components of the kinetochore complex have emerged as being the most important for transmitting the force produced by microtubule depolymerization.[39–49]

The stall force, or the amount of opposing force required to stall movement, is a useful measurement of the maximum force a molecular force generator is able to produce. The stall force of chromosome motion during anaphase was measured in the early 80s using the bending of calibrated glass needles to be about 700 pN, or about 45 pN per microtubule if one assumes 15 microtubules are attached at the kinetochore.[50] The theoretical maximum force a single microtubule can produce by depolymerization has been estimated to be about 75 pN per layer of dimer subunits removed.[51] This calculation is based on the conformational wave model of kinetochore microtubule tracking, in which the change in conformation of protofilaments from straight to curved during microtubule depolymerization exerts a power stroke that can perform work[52] (Figure 1(b)). The other prominent model for tracking of depolymerizing microtubules by the kinetochore is known as biased diffusion (Figure 1(c)), in which the kinetochore quickly binds and unbinds the microtubule though many attachments, and the energetic favorability of kinetochore components binding to the microtubule is sufficient for it to track the depolymerizing tip.[53] These two mechanisms have been investigated for decades, and some combination of the two may be operating.[10]

The *Saccharomyces cerevisiae* kinetochore protein complex Dam1 assembles *in vitro* into a ring-shaped structure that encircles microtubules, a geometry that fits well with the conformational wave model (Figure 1(b)). This assembly has been shown to track depolymerizing microtubules.[44] The average amount of force the isolated Dam1 complex is able to capture from microtubule depolymerization *in vitro* has been measured at about 2 pN, with a maximum of about 5 pN.[43,45] The addition of flexible oligopeptide tethers to the Dam1 ring allows the complex to transmit maximum pulling forces 25 pN or more, with an average of ~9 pN.[47] The fibrous Ndc80 heterotetramer could conceivably perform this function in yeast. Dam1 has been shown to recruit Ndc80 to microtubule ends, conferring to it the ability to track microtubule tips.[46,54] Ndc80 and Dam1 together are able to capture an average force of ~4.5 pN. In addition, Ndc80 and Dam1 together seem to promote rescue when placed under tension.[54]

Dam1 has only been found in fungi.[55] However, the spindle and kinetochore associated 1 (Ska1) complex may perform a similar function to Dam1 in humans.[56] Disruption of components of this complex, which comprises the proteins Ska1, Ska2, and Ska3, has been reported to cause checkpoint-mediated mitotic arrest and defects in chromatid separation.[39,40,57] Some have argued that the main role of Ska1 is to promote recruitment of protein phosphatase 1, a protein that helps initiate anaphase by stabilizing kinetochore–microtubule attachments.[58,59] Like Dam1 in yeast, Ska1 complexes with Ndc80 and gives Ndc80 the ability to track depolymerizing microtubule ends.[41,42] However, the force this complex is able to produce has not been measured. MCAK, a kinesin-like protein that also localizes to the centromere during mitosis, has been reported to induce microtubule depolymerization and cooperatively capture forces of ~1 pN per MCAK molecule *in vitro*.[34]

Motor Force Generation

Microtubules are associated with two classes of processive motors, dyneins and kinesins. These motors 'walk' directionally along microtubules. Dynein generally walks toward the minus end and kinesins toward the plus end (although dynein can take backward steps toward the plus end[60] and members of the kinesin-14 family are minus end-directed).[61] In addition to the vital functions of microtubule motors in the cell, which include cargo transport, organization of the mitotic spindle, and powering cilia and flagella, microtubule motors have possible future applications in the design of nanoscale devices—for example, to directionally transport molecules of interest toward a sensor or against flow in a microfluidic device or to provide the force for fluid flows in the pL/s range.[62,63] Transport in these devices has been accomplished by gliding microtubules decorated with linkers for molecular cargo on a substrate coated with kinesins. The microtubules then act as shuttles to deliver the cargo molecule to a desired location.[64–66]

Cytoplasmic dynein has received a great deal of attention in recent years in relation to microtubule-based force generation in cells. It belongs to the AAA

+ family of proteins and contains their distinctive ATPase ring-shaped structure in its two identical motor domains. These two motor domains are attached to each other by a linker domain, which also binds to protein complexes such as dynactin that allow dynein to transport cargo. Cytoplasmic dynein should not be confused with axonemal dynein, which is responsible for the beating of flagella and cilia but does not transport cargo. The stall force of individual dynein motors has been a subject of debate. Some groups report values between 6 and 8 pN, with processivity toward the plus end at higher loads.[60,67–69] However, other researchers report stall forces of ~1 pN and argue that multiple dynein motors act in unison to achieve larger forces.[70,71]

The other major processive motor associated with microtubules is kinesin-1. Kinesins share structural similarities with myosin which suggest a common evolutionary origin, with two heavy chains forming the motor domain and two light chains forming a coiled-coil stalk terminating in a globular cargo-binding domain.[72] Like dynein, kinesin is responsible for transporting several types of cargo in the cell, but in the opposite direction. The stall force of kinesin-1 has been measured to be ~5.5–7 pN, with smaller stall forces of ~2–7 pN measured *in vivo*.[71,73] To varying degrees, these motors are important to many cellular processes and the positioning of several structures within the cell, such as the Golgi apparatus, the mitotic spindle, and the centrosome.[23,74–81] Dynein is generally more important in the positioning of large subcellular structures. However, one exception to this is during development of skeletal muscle tissue, where both motors are required to correctly disperse and position nuclei in multinucleate myotubes but kinesin-1 seems to affect the process to a higher extent.[82–84]

One effect of microtubule motor forces that can be seen in cells is the transient bending and shape fluctuations of microtubules. In cells, microtubules bend on micron-length scales despite having a persistence length on the scale of millimeters. Much work has been done to elucidate the forces that are responsible for these deviations in microtubule shape. Microtubules have been repeatedly observed to form short-wavelength buckles near the boundary of adherent cells,[85–87] suggesting compressive loads due to polymerization. Actin retrograde flow from the lamella has also been shown to translate and buckle microtubules over relatively long timescales (0.4 μm/min).[88] In living cells, microtubules buckle at shorter wavelengths than observed *in vitro*, and one mechanism proposed for this is microtubule interaction with and reinforcement by the surrounding cytoskeleton. Qualitatively similar bending behavior is observed on the macroscale with a plastic rod submerged in an elastic gel.[86] In addition, microtubules embedded in a reconstituted actin cytoskeleton with myosin II motors can be bent by contraction of the actin network.[89]

Tracking motion of microtubules along their length in epithelial cells has uncovered that most microtubules that bend near the cell periphery as well as away from the periphery under the nucleus bend by lengthwise motion of a portion of the microtubule in the direction of the plus end, causing buckling.[85,90] This observation strongly suggests that a different mechanism is at play than either polymerization against a boundary or actin retrograde flow, as these mechanisms would both cause motion of a microtubule toward the minus end rather than toward the plus end. Actin retrograde flow is too slow to be causing this bending, which occurs over only a few seconds, and actomyosin contractility would not be expected to cause microtubule translation primarily toward the plus end during bending.

Microtubules in *in vitro* gliding assays have long been known to form similar bends to those seen *in vivo*. In these assays, microtubule motors coated onto a substrate process along reconstituted microtubules, causing them to 'glide' along the substrate.[91] Some of the motors in these assays are nonfunctional and act as passive crosslinkers between the substrate and the microtubule. When another motor processes along a microtubule bound to a dead motor, the microtubule buckles under the produced force. A similar process could be taking place *in vivo* and the motor most likely to cause such a force is cytoplasmic dynein, which due to its directionality toward the minus end exerts a plus end-directed force on the microtubule.[85]

Observation of microtubule motion has shown that dynein is indeed responsible for the biased directional motion during microtubule bend formation.[90] To directly observe movement of a microtubule along its length, fluorescent microtubules were photobleached at multiple locations to give them a dashed appearance. These nonfluorescent patches acted as markers to keep track of microtubule motion. Bending occurred when a portion of the microtubule translated persistently in one direction. This motile portion of the microtubule buckled against a stationary portion, much as the hind portion of an inchworm 'buckles' against the stationary anterior portion as it moves. Normally, a majority of this motion occurred toward the plus end, but upon dynein inhibition, this bias in direction was lost and less bends formed overall. This indicates that while

microtubule bending can occur by multiple mechanisms, dynein likely is responsible for a majority of this behavior.

Dynein activity also affects growth trajectories of microtubules.[92] Experiments charting the trajectories of +TIP protein end-binding 1 (EB1) have shown that when dynein is inhibited in fibroblasts, microtubules tend to grow along a straighter path. A model for this effect of dynein was put forth in which dynein motors attached to stiff anchor points at their cargo ends. Motion of the motor and/or the anchor point (as might be caused by myosin contraction) causes a force to be applied to the microtubule that has both a tangential and lateral component, and the lateral component causes deflection of the microtubule plus end as it grows.[92] Dynein-driven microtubule bending near the plus end can also change the growth trajectory of a microtubule. In this mechanism, tangential microtubule motion toward the plus end buckles the microtubule against a crosslinked stationary site just behind the growing plus end. As the microtubule buckles, the microtubule segment between the stationary site and the plus end rotates until it grows long enough to be stabilized by frictional forces from interaction with the surrounding cell environment.[90]

In addition to altering the shapes of microtubules, dynein has been found to generate forces upon the nucleus to rotate it. The nucleus rotates in stationary[79,93,94] as well as migrating cells.[74] Fibroblasts closing a wound in a monolayer exhibit nuclear rotation, which is reduced upon depletion of dynein heavy chain.[74] Overexpression of $p150^{Glued}$(217–548), a construct that competitively inhibits dynein–dynactin binding,[95] in these cells caused a drastic reduction in nuclear rotation.[79] A model was developed in which dynein motors bound to microtubules at the nuclear periphery transiently bind to the nucleus, pulling it toward the microtubule's minus end. Because of the geometry of the microtubule aster, this pulling force by dynein exerts a torque on the nucleus. A key prediction of the model is that nuclear rotation should depend on the distance of the nucleus from the centrosome, decreasing when the centrosome is closer to the centroid of the nucleus, and this prediction has been confirmed experimentally.[79]

There are several mechanisms by which dynein can position the microtubule aster and associated cellular structures, such as the centrosome or mitotic spindle. In budding yeast, the spindle is pulled toward the budding site by dynein localized to the cortex of the bud. Astral microtubules are captured by the cortical dynein and bend at their plus ends, pulled along the cell cortex by multiple dynein motors.[81] Additionally, during anaphase in budding yeast, the spindle is pulled by microtubule shrinkage at the bud cortex in a process which is dependent on dynein. In this process, the proposed role of dynein is to couple microtubules to the cortex during depolymerization induced by the kinesin-8 Kip3p.[96] An *in vitro* study in which microtubules grown in microchambers with walls decorated with dynein has shown that dynein is able to maintain an interaction with shrinking microtubules in an end-on fashion, causing pulling forces of up to 5 pN.[97]

Alternatively, in adherent cells on a substrate, microtubules could be pulled along their length by dynein motors located at the bottom surface of the cell, in an arrangement similar to an *in vitro* gliding assay.[98] When new minus ends are created by laser ablation of an existing microtubule, the curvature of the microtubule near the new minus end increases in a dynein-dependent manner, which suggests that dynein pulls along the microtubule length in cells.[23] There is evidence of lengthwise forces being exerted along the contour of microtubules by dynein bound to the cell cortex or other relatively stiff structures in the cell.[23,85,90] Dynein is necessary for proper positioning of the centrosome in migrating fibroblasts during wound healing[99] and in micropatterned endothelial cells.[23] In simulations, lengthwise dynein pulling forces were able to center a microtubule aster in a relatively large square geometry (40 μm × 40 μm), while polymerization forces were insufficient for centering in this geometry owing to the large frictional resistance to microtubule motion.[23] Polymerization forces are sufficient for centering in smaller geometries *in vitro*[100] likely because the frictional resistance to motion in water is far lower.

MICROTUBULES AS TARGETS IN DISEASE

Microtubules have long been an important target for cancer treatment owing to their pivotal role in mitosis. In anaphase, microtubules attached to chromosomes shorten, exerting a mechanical force on chromosomes which pulls them apart. Many chemotherapeutic drugs seek to arrest the cell cycle by preventing the cell from entering anaphase.[101,102] Vinca alkaloids such as vinblastine are classified as microtubule destabilizers and inhibit microtubule polymerization when present in a high enough concentration.[103] Conversely, taxanes such as paclitaxel and docetaxel stabilize microtubules, promoting polymerization at higher concentrations.[101,104] However, at lower, clinically relevant concentrations, both classes of drug act to suppress microtubule dynamic instability—a function that is

thought to be their primary mechanism of antimitotic activity.[101] Microtubules have been shown to be several times more dynamic during mitosis than during interphase.[105,106] Upon treatment with microtubule-targeting drugs, these dynamics are disrupted, the spindle-assembly checkpoint is activated, and the cell cycle is arrested at the metaphase–anaphase transition.[107]

While it is clear that paclitaxel and other drugs are able to induce cell death in tumors, the exact mechanism remains unknown. Prolonged mitotic arrest can trigger apoptosis due to depletion of RNA transcripts, as the condensed chromosomes present during mitosis cannot be transcribed.[108] However, cells are sometimes able to exit mitosis under unfavorable conditions in a process called mitotic slippage.[109] Paclitaxel induces apoptosis at a higher rate in cancer cell lines than in untransformed epithelial cells[110] and at a higher rate in culture than in human xenographs in mice.[111] Furthermore, while apoptosis is dependent on drug concentration, it does not seem to be linked to the duration of mitotic arrest, indicating that disruption of microtubule functions during interphase likely plays a role in drug efficacy. Drugs targeting microtubules can cause serious side effects owing to their importance in the nervous system, such as peripheral neuropathy and myelosuppression. For these reasons, it is important to understand the behavior and functions of microtubules in interphase cells.

Microtubule motors are also increasingly being investigated for cancer treatment. Drugs targeting various kinesins continue to be explored for their antimitotic activity, as they could potentially be more specific to mitotic cells than traditional antimitotics.[112] However, their inability to produce very favorable clinical results thus far may mean that antimitotic activity alone is insufficient to treat tumors in patients.[113] Additionally, cytoplasmic dynein has been implicated in various neurodegenerative disorders. For example, some cases of inherited amyotropic lateral sclerosis have been linked to mutations in Cu/Zn superoxide dismutase (SOD1), and there is evidence suggesting that the pathological mechanism relates to a gain of interaction of SOD1 with dynein, interfering with normal axonal transport.[114] Various studies with animal models have indirectly linked members of the dynein–dynactin complex to several other neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease.[115–119]

CONCLUSION

In order to fully understand microtubule function in normal cells and in disease, it is important to understand how microtubules generate and respond to forces. The cycles of polymerization and depolymerization inherent to microtubules are regulated and harnessed by cells to position cellular structures such as the mitotic spindle and nucleus in yeast. Depolymerization of microtubules is vital to the separation of chromosomes during mitosis, and helps in positioning the yeast mitotic spindle as well. The microtubule-associated molecular motors dynein and kinesin also play important roles. In addition to transporting cargo, dynein can center microtubule asters by lengthwise pulling as well as capture and pull microtubules from the cortex. Dynein also influences how microtubules explore space as they grow and exerts forces which bend microtubules. Researchers have come up with creative ways to assay these forces, using tools such as fluorescence microscopy, optical traps, microfabrication, and laser ablation. In the future, more work must be done to determine how microtubule forces are affected in disease states.

REFERENCES

1. Desai A, Mitchison TJ. Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* 1997, 13:83–117.
2. Evans L, Mitchison T, Kirschner M. Influence of the centrosome on the structure of nucleated microtubules. *J Cell Biol* 1985, 100:1185–1191.
3. Howard J, Hyman AA. Dynamics and mechanics of the microtubule plus end. *Nature* 2003, 422:753–758.
4. Seetapun D, Castle BT, McIntyre AJ, Tran PT, Odde DJ. Estimating the microtubule GTP cap size in vivo. *Curr Biol* 2012, 22:1681–1687.
5. Dimitrov A, Quesnoit M, Moutel S, Cantaloube I, Poüs C, Perez F. Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. *Science* 2008, 322:1353–1356.
6. Tropini C, Roth EA, Zanic M, Gardner MK, Howard J. Islands containing slowly hydrolyzable GTP analogs promote microtubule rescues. *PLoS One* 2012, 7:e30103.
7. Jiang K, Hua S, Mohan R, Grigoriev I, Yau KW, Liu Q, Katrukha EA, Altelaar AF, Heck AJ, Hoogenraad CC, et al. Microtubule minus-end

stabilization by polymerization-driven CAMSAP deposition. *Dev Cell* 2014, 28:295–309.

8. Dammermann A, Desai A, Oegema K. The minus end in sight. *Curr Biol* 2003, 13:R614–R624.
9. Akhmanova A, Steinmetz MO. Control of microtubule organization and dynamics: two ends in the limelight. *Nat Rev Mol Cell Biol* 2015, 16:711–726.
10. Asbury CL, Tien JF, Davis TN. Kinetochores' gripping feat: conformational wave or biased diffusion? *Trends Cell Biol* 2011, 21:38–46.
11. Gittes F, Mickey B, Nettleton J, Howard J. Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *J Cell Biol* 1993, 120:923–934.
12. Marko J, Siggia E. Stretching DNA. *Macromolecules* 1995, 28:8759–8770.
13. Brinkers S, Dietrich H, de Groote F, Young I, Rieger B. The persistence length of double stranded DNA determined using dark field tethered particle motion. *J Chem Phys* 2009, 130:215105. doi: 10.1063/1.3142699.
14. Etienne-Manneville S, Schekman R. Microtubules in cell migration. *Annu Rev Cell Dev Biol* 2013, 29:471–499.
15. Peskin CS, Odell GM, Oster GF. Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys J* 1993, 65:316–324.
16. Mogilner A, Oster G. The polymerization ratchet model explains the force-velocity relation for growing microtubules. *Eur Biophys J* 1999, 28:235–242.
17. Janson ME, de Dood ME, Dogterom M. Dynamic instability of microtubules is regulated by force. *J Cell Biol* 2003, 161:1029–1034.
18. Dogterom M, Yurke B. Measurement of the force-velocity relation for growing microtubules. *Science* 1997, 278:856–860.
19. Dogterom M, Kerssemakers JW, Romet-Lemonne G, Janson ME. Force generation by dynamic microtubules. *Curr Opin Cell Biol* 2005, 17:67–74.
20. Drummond DR, Cross RA. Dynamics of interphase microtubules in *Schizosaccharomyces pombe*. *Curr Biol* 2000, 10:766–775.
21. Tran PT, Marsh L, Doye V, Inoué S, Chang F. A mechanism for nuclear positioning in fission yeast based on microtubule pushing. *J Cell Biol* 2001, 153:397–411.
22. Garzon-Coral C, Fantana HA, Howard J. A force-generating machinery maintains the spindle at the cell center during mitosis. *Science* 2016, 352:1124–1127.
23. Wu J, Misra G, Russell RJ, Ladd AJ, Lele TP, Dickinson RB. Effects of dynein on microtubule mechanics and centrosome positioning. *Mol Biol Cell* 2011, 22:4834–4841.
24. Georgatos SD, Pyrpasopoulou A, Theodoropoulos PA. Nuclear envelope breakdown in mammalian cells involves stepwise lamina disassembly and microtubule-drive deformation of the nuclear membrane. *J Cell Sci* 1997, 110(Pt 17):2129–2140.
25. Gerlitz G, Reiner O, Bustin M. Microtubule dynamics alter the interphase nucleus. *Cell Mol Life Sci* 2013, 70:1255–1268.
26. Goldberg DJ, Burmeister DW. Microtubule-based filopodium-like protrusions form after axotomy. *J Neurosci* 1992, 12:4800–4807.
27. Al-Bassam J, Chang F. Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP. *Trends Cell Biol* 2011, 21:604–614.
28. Al-Bassam J, Kim H, Brouhard G, van Oijen A, Harrison SC, Chang F. CLASP promotes microtubule rescue by recruiting tubulin dimers to the microtubule. *Dev Cell* 2010, 19:245–258.
29. Komarova YA, Akhmanova AS, Kojima S, Galjart N, Borisy GG. Cytoplasmic linker proteins promote microtubule rescue in vivo. *J Cell Biol* 2002, 159:589–599.
30. Gupta KK, Joyce MV, Slabbekoorn AR, Zhu ZC, Paulson BA, Boggess B, Goodson HV. Probing interactions between CLIP-170, EB1, and microtubules. *J Mol Biol* 2010, 395:1049–1062.
31. Arnal I, Heichette C, Diamantopoulos GS, Chrétien D. CLIP-170/tubulin-curved oligomers coassemble at microtubule ends and promote rescues. *Curr Biol* 2004, 14:2086–2095.
32. Dixit R, Levy JR, Tokito M, Ligon LA, Holzbaur EL. Regulation of dynactin through the differential expression of p150Glued isoforms. *J Biol Chem* 2008, 283:33611–33619.
33. Lazarus JE, Moughamian AJ, Tokito MK, Holzbaur EL. Dynactin subunit p150(Glued) is a neuron-specific anti-catastrophe factor. *PLoS Biol* 2013, 11:e1001611.
34. Oguchi Y, Uchimura S, Ohki T, Mikhailenko SV, Ishiwata S. The bidirectional depolymerizer MCAK generates force by disassembling both microtubule ends. *Nat Cell Biol* 2011, 13:846–852.
35. Ganguly A, Yang H, Cabral F. Overexpression of mitotic centromere-associated Kinesin stimulates microtubule detachment and confers resistance to paclitaxel. *Mol Cancer Ther* 2011, 10:929–937.
36. Sanhaji M, Friel CT, Wordeman L, Louwen F, Yuan J. Mitotic centromere-associated kinesin (MCAK): a potential cancer drug target. *Oncotarget* 2011, 2:935–947.
37. Su X, Ohi R, Pellman D. Move in for the kill: motile microtubule regulators. *Trends Cell Biol* 2012, 22:567–575.
38. Pesenti ME, Weir JR, Musacchio A. Progress in the structural and functional characterization of kinetochores. *Curr Opin Struct Biol* 2016, 37:152–163.

39. Daum JR, Wren JD, Daniel JJ, Sivakumar S, McAvoy JN, Potapova TA, Gorbsky GJ. Ska3 is required for spindle checkpoint silencing and the maintenance of chromosome cohesion in mitosis. *Curr Biol* 2009, 19:1467–1472.
40. Gaitanos TN, Santamaria A, Jeyaprakash AA, Wang B, Conti E, Nigg EA. Stable kinetochore-microtubule interactions depend on the Ska complex and its new component Ska3/C13Orf3. *EMBO J* 2009, 28:1442–1452.
41. Schmidt JC, Arthanari H, Boeszoermenyi A, Dashkevich NM, Wilson-Kubalek EM, Monnier N, Markus M, Oberer M, Milligan RA, Bathe M, et al. The kinetochore-bound Ska1 complex tracks depolymerizing microtubules and binds to curved protofilaments. *Dev Cell* 2012, 23:968–980.
42. Welburn JP, Grishchuk EL, Backer CB, Wilson-Kubalek EM, Yates JR, Cheeseman IM. The human kinetochore Ska1 complex facilitates microtubule depolymerization-coupled motility. *Dev Cell* 2009, 16:374–385.
43. Asbury CL, Gestaut DR, Powers AF, Franck AD, Davis TN. The Dam1 kinetochore complex harnesses microtubule dynamics to produce force and movement. *Proc Natl Acad Sci USA* 2006, 103:9873–9878.
44. Westermann S, Wang HW, Avila-Sakar A, Drubin DG, Nogales E, Barnes G. The Dam1 kinetochore ring complex moves processively on depolymerizing microtubule ends. *Nature* 2006, 440:565–569.
45. Grishchuk EL, Efremov AK, Volkov VA, Spiridonov IS, Gudimchuk N, Westermann S, Drubin D, Barnes G, McIntosh JR, Ataullakhanov FI. The Dam1 ring binds microtubules strongly enough to be a processive as well as energy-efficient coupler for chromosome motion. *Proc Natl Acad Sci USA* 2008, 105:15423–15428.
46. Lampert F, Hornung P, Westermann S. The Dam1 complex confers microtubule plus end-tracking activity to the Ndc80 kinetochore complex. *J Cell Biol* 2010, 189:641–649.
47. Volkov VA, Zaytsev AV, Gudimchuk N, Grissom PM, Gintsburg AL, Ataullakhanov FI, McIntosh JR, Grishchuk EL. Long tethers provide high-force coupling of the Dam1 ring to shortening microtubules. *Proc Natl Acad Sci USA* 2013, 110:7708–7713.
48. Umbreit NT, Miller MP, Tien JF, Ortolá JC, Gui L, Lee KK, Biggins S, Asbury CL, Davis TN. Kinetochores require oligomerization of Dam1 complex to maintain microtubule attachments against tension and promote biorientation. *Nat Commun* 2014, 5:4951.
49. Powers AF, Franck AD, Gestaut DR, Cooper J, Gracyzk B, Wei RR, Wordeman L, Davis TN, Asbury CL. The Ndc80 kinetochore complex forms load-bearing attachments to dynamic microtubule tips via biased diffusion. *Cell* 2009, 136:865–875.
50. Nicklas RB. Measurements of the force produced by the mitotic spindle in anaphase. *J Cell Biol* 1983, 97:542–548.
51. Molodtsov MI, Grishchuk EL, Efremov AK, McIntosh JR, Ataullakhanov FI. Force production by depolymerizing microtubules: a theoretical study. *Proc Natl Acad Sci USA* 2005, 102:4353–4358.
52. Koshland DE, Mitchison TJ, Kirschner MW. Polewards chromosome movement driven by microtubule depolymerization in vitro. *Nature* 1988, 331:499–504.
53. Hill TL. Theoretical problems related to the attachment of microtubules to kinetochores. *Proc Natl Acad Sci USA* 1985, 82:4404–4408.
54. Tien JF, Umbreit NT, Gestaut DR, Franck AD, Cooper J, Wordeman L, Gonen T, Asbury CL, Davis TN. Cooperation of the Dam1 and Ndc80 kinetochore complexes enhances microtubule coupling and is regulated by aurora B. *J Cell Biol* 2010, 189:713–723.
55. McIntosh JR, Volkov V, Ataullakhanov FI, Grishchuk EL. Tubulin depolymerization may be an ancient biological motor. *J Cell Sci* 2010, 123(Pt 20):3425–3434.
56. Hanisch A, Silljé HH, Nigg EA. Timely anaphase onset requires a novel spindle and kinetochore complex comprising Ska1 and Ska2. *EMBO J* 2006, 25:5504–5515.
57. Theis M, Slabicki M, Junqueira M, Paszkowski-Rogacz M, Sontheimer J, Kittler R, Heninger AK, Glatter T, Kruusmaa K, Poser I, et al. Comparative profiling identifies C13orf3 as a component of the Ska complex required for mammalian cell division. *EMBO J* 2009, 28:1453–1465.
58. Sivakumar S, Janczyk P, Qu Q, Brautigam C, Stukenberg P, Yu H, Gorbsky G. The human SKA complex drives the metaphase-anaphase cell cycle transition by recruiting protein phosphatase 1 to kinetochores. *Elife* 2016, 5.
59. Liu D, Vleugel M, Backer CB, Hori T, Fukagawa T, Cheeseman IM, Lampson MA. Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes Aurora B kinase. *J Cell Biol* 2010, 188:809–820.
60. Gennerich A, Carter AP, Reck-Peterson SL, Vale RD. Force-induced bidirectional stepping of cytoplasmic dynein. *Cell* 2007, 131:952–965.
61. Cross RA. Kinesin-14: the roots of reversal. *BMC Biol* 2010, 8:107.
62. Hess H. Engineering applications of biomolecular motors. *Annu Rev Biomed Eng* 2011, 13:429–450.

63. Bull JL, Hunt AJ, Meyhöfer E. A theoretical model of a molecular-motor-powered pump. *Biomed Microdevices* 2005, 7:21–33.
64. Kim T, Cheng LJ, Kao MT, Hasselbrink EF, Guo L, Meyhöfer E. Biomolecular motor-driven molecular sorter. *Lab Chip* 2009, 9:1282–1285.
65. Nitta T, Hess H. Dispersion in active transport by kinesin-powered molecular shuttles. *Nano Lett* 2005, 5:1337–1342.
66. Schmidt C, Vogel V. Molecular shuttles powered by motor proteins: loading and unloading stations for nanocargo integrated into one device. *Lab Chip* 2010, 10:2195–2198.
67. Reck-Peterson S, Yildiz A, Carter A, Gennerich A, Zhang N, Vale R. Single-molecule analysis of dynein processivity and stepping behavior. *Cell* 2006, 126:335–348.
68. Toba S, Watanabe TM, Yamaguchi-Okimoto L, Toyoshima YY, Higuchi H. Overlapping hand-over-hand mechanism of single molecular motility of cytoplasmic dynein. *Proc Natl Acad Sci USA* 2006, 103:5741–5745.
69. Sims P, Xie X. Probing dynein and kinesin stepping with mechanical manipulation in a living cell. *Chemphyschem* 2009, 10:1511–1516.
70. Rai AK, Rai A, Ramaiya AJ, Jha R, Mallik R. Molecular adaptations allow dynein to generate large collective forces inside cells. *Cell* 2013, 152:172–182.
71. Blehm BH, Schroer TA, Trybus KM, Chemla YR, Selvin PR. In vivo optical trapping indicates kinesin's stall force is reduced by dynein during intracellular transport. *Proc Natl Acad Sci USA* 2013, 110:3381–3386.
72. Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD. Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. *Nature* 1996, 380:550–555.
73. Schnitzer M, Visscher K, Block S. Force production by single kinesin motors. *Nat Cell Biol* 2000, 2:718–723.
74. Levy JR, Holzbaur EL. Dynein drives nuclear rotation during forward progression of motile fibroblasts. *J Cell Sci* 2008, 121(Pt 19):3187–3195.
75. Yadav S, Linstedt AD. Golgi positioning. *Cold Spring Harb Perspect Biol* 2011, 3.
76. Roux KJ, Crisp ML, Liu Q, Kim D, Kozlov S, Stewart CL, Burke B. Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization. *Proc Natl Acad Sci USA* 2009, 106:2194–2199.
77. McNally FJ. Mechanisms of spindle positioning. *J Cell Biol* 2013, 200:131–140.
78. Agircan FG, Schiebel E, Mardin BR. Separate to operate: control of centrosome positioning and separation. *Philos Trans R Soc Lond B Biol Sci* 2014, 369:20130461.
79. Wu J, Lee KC, Dickinson RB, Lele TP. How dynein and microtubules rotate the nucleus. *J Cell Physiol* 2011, 226:2666–2674.
80. Fridolfsson HN, Starr DA. Kinesin-1 and dynein at the nuclear envelope mediate the bidirectional migrations of nuclei. *J Cell Biol* 2010, 191:115–128.
81. Adames NR, Cooper JA. Microtubule interactions with the cell cortex causing nuclear movements in *Saccharomyces cerevisiae*. *J Cell Biol* 2000, 149:863–874.
82. Wilson MH, Holzbaur EL. Nesprins anchor kinesin-1 motors to the nucleus to drive nuclear distribution in muscle cells. *Development* 2015, 142:218–228.
83. Metzger T, Gache V, Xu M, Cadot B, Folker ES, Richardson BE, Gomes ER, Baylies MK. MAP and kinesin-dependent nuclear positioning is required for skeletal muscle function. *Nature* 2012, 484:120–124.
84. Cadot B, Gache V, Vasyutina E, Falcone S, Birchmeier C, Gomes ER. Nuclear movement during myotube formation is microtubule and dynein dependent and is regulated by Cdc42, Par6 and Par3. *EMBO Rep* 2012, 13:741–749.
85. Bicek AD, Tüzel E, Demtchouk A, Uppalapati M, Hancock WO, Kroll DM, Odde DJ. Anterograde microtubule transport drives microtubule bending in LLC-PK1 epithelial cells. *Mol Biol Cell* 2009, 20:2943–2953.
86. Brangwynne CP, MacKintosh FC, Kumar S, Geisse NA, Talbot J, Mahadevan L, Parker KK, Ingber DE, Weitz DA. Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement. *J Cell Biol* 2006, 173:733–741.
87. Wang N, Naruse K, Stamenović D, Fredberg JJ, Mijailovich SM, Tolić-Nørrelykke IM, Polte T, Mannix R, Ingber DE. Mechanical behavior in living cells consistent with the tensegrity model. *Proc Natl Acad Sci USA* 2001, 98:7765–7770.
88. Waterman-Storer CM, Salmon ED. Actomyosin-based retrograde flow of microtubules in the lamella of migrating epithelial cells influences microtubule dynamic instability and turnover and is associated with microtubule breakage and treadmilling. *J Cell Biol* 1997, 139:417–434.
89. Brangwynne CP, Koenderink GH, Mackintosh FC, Weitz DA. Nonequilibrium microtubule fluctuations in a model cytoskeleton. *Phys Rev Lett* 2008, 100:118104.
90. Kent IA, Rane PS, Dickinson RB, Ladd AJ, Lele TP. Transient pinning and pulling: a mechanism for bending microtubules. *PLoS One* 2016, 11:e0151322.
91. Amos LA, Amos WB. The bending of sliding microtubules imaged by confocal light microscopy and negative stain electron microscopy. *J Cell Sci Suppl* 1991, 14:95–101.
92. Shekhar N, Neelam S, Wu J, Ladd AJ, Dickinson RB, Lele TP. Fluctuating motor forces bend growing microtubules. *Cell Mol Bioeng* 2013, 6:120–129.

93. Yao KT, Ellingson DJ. Observations on nuclear rotation and oscillation in Chinese hamster germinal cells in vitro. *Exp Cell Res* 1969, 55:39–42.
94. Parker KK, Brock AL, Brangwynne C, Mannix RJ, Wang N, Ostuni E, Geisse NA, Adams JC, Whitesides GM, Ingber DE. Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J* 2002, 16:1195–1204.
95. Quintyne NJ, Gill SR, Eckley DM, Crego CL, Compton DA, Schroer TA. Dynactin is required for microtubule anchoring at centrosomes. *J Cell Biol* 1999, 147:321–334.
96. Ten Hoopen R, Cepeda-García C, Fernández-Arruti R, Juanes MA, Delgehyr N, Segal M. Mechanism for astral microtubule capture by cortical Bud6p priming spindle polarity in *S. cerevisiae*. *Curr Biol* 2012, 22:1075–1083.
97. Laan L, Pavin N, Husson J, Romet-Lemonne G, van Duijn M, López MP, Vale RD, Jülicher F, Reck-Peterson SL, Dogterom M. Cortical dynein controls microtubule dynamics to generate pulling forces that position microtubule asters. *Cell* 2012, 148:502–514.
98. Zhu J, Burakov A, Rodionov V, Mogilner A. Finding the cell center by a balance of dynein and myosin pulling and microtubule pushing: a computational study. *Mol Biol Cell* 2010, 21:4418–4427.
99. Palazzo AF, Joseph HL, Chen YJ, Dujardin DL, Alberts AS, Pfister KK, Vallee RB, Gundersen GG. Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Curr Biol* 2001, 11:1536–1541.
100. Holy TE, Dogterom M, Yurke B, Leibler S. Assembly and positioning of microtubule asters in microfabricated chambers. *Proc Natl Acad Sci USA* 1997, 94:6228–6231.
101. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer* 2004, 4:253–265.
102. van Vuuren RJ, Visagie MH, Theron AE, Joubert AM. Antimitotic drugs in the treatment of cancer. *Cancer Chemother Pharmacol* 2015, 76:1101–1112.
103. Moudi M, Go R, Yien CY, Nazre M. Vinca alkaloids. *Int J Prev Med* 2013, 4:1231–1235.
104. Barbuti AM, Chen ZS. Paclitaxel through the ages of anticancer therapy: exploring its role in chemoresistance and radiation therapy. *Cancers (Basel)* 2015, 7:2360–2371.
105. Zhai Y, Kronebusch PJ, Simon PM, Borisy GG. Microtubule dynamics at the G2/M transition: abrupt breakdown of cytoplasmic microtubules at nuclear envelope breakdown and implications for spindle morphogenesis. *J Cell Biol* 1996, 135:201–214.
106. Rusan NM, Fagerstrom CJ, Yvon AM, Wadsworth P. Cell cycle-dependent changes in microtubule dynamics in living cells expressing green fluorescent protein-α tubulin. *Mol Biol Cell* 2001, 12:971–980.
107. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol* 2007, 8:379–393.
108. Blagosklonny MV. Mitotic arrest and cell fate: why and how mitotic inhibition of transcription drives mutually exclusive events. *Cell Cycle* 2007, 6:70–74.
109. Topham CH, Taylor SS. Mitosis and apoptosis: how is the balance set? *Curr Opin Cell Biol* 2013, 25:780–785.
110. Brito DA, Rieder CL. The ability to survive mitosis in the presence of microtubule poisons differs significantly between human nontransformed (RPE-1) and cancer (U2OS, HeLa) cells. *Cell Motil Cytoskeleton* 2009, 66:437–447.
111. Orth JD, Kohler RH, Foijer F, Sorger PK, Weissleder R, Mitchison TJ. Analysis of mitosis and antimitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics. *Cancer Res* 2011, 71:4608–4616.
112. Chandrasekaran G, Tátrai P, Gergely F. Hitting the brakes: targeting microtubule motors in cancer. *Br J Cancer* 2015, 113:693–698.
113. Komlodi-Pasztor E, Sackett DL, Fojo AT. Inhibitors targeting mitosis: tales of how great drugs against a promising target were brought down by a flawed rationale. *Clin Cancer Res* 2012, 18:51–63.
114. Zhang F, Ström AL, Fukada K, Lee S, Hayward LJ, Zhu H. Interaction between familial amyotrophic lateral sclerosis (ALS)-linked SOD1 mutants and the dynein complex. *J Biol Chem* 2007, 282:16691–16699.
115. Kimura N, Imamura O, Ono F, Terao K. Aging attenuates dynactin-dynein interaction: down-regulation of dynein causes accumulation of endogenous tau and amyloid precursor protein in human neuroblastoma cells. *J Neurosci Res* 2007, 85:2909–2916.
116. Wiggins LM, Kuta A, Stevens JC, Fisher EM, von Bartheld CS. A novel phenotype for the dynein heavy chain mutation Loa: altered dendritic morphology, organelle density, and reduced numbers of trigeminal motoneurons. *J Comp Neurol* 2012, 520:2757–2773.
117. Hafezparast M, Klocke R, Ruhrberg C, Marquardt A, Ahmad-Annuar A, Bowen S, Lalli G, Witherden AS, Hummerich H, Nicholson S, et al. Mutations in dynein link motor neuron degeneration to defects in retrograde transport. *Science* 2003, 300:808–812.
118. Farrer MJ, Hulihan MM, Kachergus JM, Dächsel JC, Stoessl AJ, Grantier LL, Calne S, Calne DB, Lechevalier B, Chapon F, et al. DCTN1 mutations in Perry syndrome. *Nat Genet* 2009, 41:163–165.
119. Chen XJ, Xu H, Cooper HM, Liu Y. Cytoplasmic dynein: a key player in neurodegenerative and neurodevelopmental diseases. *Sci China Life Sci* 2014, 57:372–377.