Cell. Mol. Life Sci. 62 (2005) 3039–3056
1420-682X/05/243039-18
DOI 10.1007/s00018-005-5330-x

Cellular and Molecular Life Sciences

Review

Understanding microtubule dynamics for improved cancer therapy

S. Honore, E. Pasquier and D. Braguer*

FRE-CNRS 2737, UFR Pharmacie, 27 bd Jean Moulin, 13005 Marseille (France), Fax: +33 4 91 83 56 35, e-mail: diane.braguer@pharmacie.univ-mrs.fr

Received 22 July 2005; received after revision 8 September 2005; accepted 12 September 2005
Online First 28 November 2005

Abstract. Microtubules (MTs), key components of the cytoskeleton, are dynamic polymers of tubulin that form a well-organized network of polarized tube filaments. MT dynamics are highly regulated both spacially and temporally by several MT-related proteins, themselves regulated by several kinases and phosphatases via signaling cascades, and also by coordinated interactions with actin cytoskeleton and adhesion sites. Regulation of MT dynamics is crucial for mitosis, cell migration, cell signaling and trafficking. MT-targeted drugs (MTDs), which constitute a major anticancer drug family with antimitotic and antiangiogenic properties, inhibit tumor progression mainly by altering MT dynamics in both cancer and endothelial cells. Identification of proteins regulating the MT network will lead to a better understanding of tumor progression regulators and will be helpful in improving cancer therapy.

Key words: Microtubule dynamics; tumor progression; microtubule-targeted drugs; mitosis; migration; cytoskeleton.

Introduction

Microtubules (MTs) are dynamic polymers of α/β tubulin heterodimers, arranged head to tail to form hollow tubes of 25 nm diameter, up to several micrometers long. The two MT poles differ in their dynamic properties, the plus end being more dynamic than the minus end. In eukaryotic cells, MTs form a well-organized network in which the minus ends are generally anchored at the centrosome or MT organizing center (MTOC) whereas the free plus ends probe the cytoplasm, in a search-and-capture-process, to reach specific targets. MT dynamics are tightly regulated both spatially and temporally. This regulation involves nucleotides, MT-associated proteins (MAPs), kinases and phosphatases as well as coordinated interactions with other cytoskeletal components, such as actin filaments, and integrin-containing adhesion sites. This intrinsic dynamic behaviour and its regulation are crucial for MT-specific functions such as cell shape maintenance, cell division, cell signaling, intracellular vesicules and organelle transport, cell polarity and locomotion. Disturbance of MT dynamics regulation thus has critical consequences for cell fate and represents a potent therapeutic target, especially in oncology.

Here, we present a review of the most recent studies focusing on the regulation of MT dynamics, investigating their involvement in mitosis and migration, and the effects of MT-targeted anticancer drugs (MTDs) on these dynamic properties during tumor growth and neoangiogenesis.

* Corresponding author.

Microtubule dynamics

Dynamic instability and treadmilling of microtubules

Polymerization of MTs occurs by a nucleation-elongation mechanism in which the formation of a short MT 'nucleus' is followed by elongation of the MT at its ends by reversible, non-covalent addition of α/β tubulin dimers. In 1984, Mitchison and Kirschner [1, 2] proposed the dynamic instability model of MT assembly, in which individual MTs exist either in an elongation state or a rapidly shortening state, with abrupt and apparently random transitions between these two states. Several studies performed *in vitro*, using purified tubulin [3, 4], or in living cells [5] have confirmed this mechanism of MT assembly/disassembly. MTs are thus governed by an intrinsic property involving repetitive spurts of shortening from their plus ends, followed by periods of polymerization. This non-equilibrium behavior is based on the binding and hydrolysis of GTP at the nucleotide exchangeable site (E-site) in β tubulin. Only dimers that have GTP in their E-site can polymerize. This nucleotide is then hydrolyzed and becomes non-exchangeable. The GTP cap model proposes that the GDP-tubulin core of MT is stabilized at the plus end by a layer of GTP-tubulin subunits that may act to maintain association between protofilaments [6, 7]. When this cap is stochastically lost, the protofilaments peel outward and the MT rapidly depolymerizes. Although both MT ends can either grow or shorten, the changes in length at the plus end are much greater than at the minus end.

MTs exhibit another important dynamic behavior called treadmilling or flux. It corresponds to a polymer mass steady state resulting from the net growth at one MT end and the net shortening at the opposite end [8]. In other words, treadmilling is a process by which tubulin subunits continuously flux from one end of the polymer to the other, due to net differences in the critical subunit concentrations at the opposite MT ends.

Both dynamic instability and treadmilling can be produced as distinct phenomena *in vitro* using purified tubulin, and they have also been observed in living cells [8, 9]. These dynamic properties have been recently reviewed [10, 11].

Microtubule dynamics analysis in living cells

Dynamic instability is characterized by four main parameters: the rates of MT growth and shortening, the frequency of transition from growth or pause to shortening (called catastrophe) and the frequency of transition from shortening to growth or pause (called rescue). Periods of pause are the condition in which changes in MT length are drastically attenuated or completely stopped. The parameter called 'dynamicity' is used to describe the overall rate of tubulin subunits exchange at MT ends [12].

Several techniques can be used to measure MT dynamics in living cells. Individual fluorescent MTs can be readily visualized in thin peripheral regions of cells after microinjection of fluorescent tubulin (e. g. rhodamine-labeled tubulin) or by expression of GFP-labeled tubulin. MT growth and shortening dynamics are recorded by time-lapse video microscopy. To determine how MT length changes with time, individual MTs ends are traced by a cursor on succeeding time-lapse frames. Rate, length and duration of growth and shortening events are calculated from the recorded x-y positions of the MT ends.

While it was possible to measure dynamic instability of individual MTs at the cell periphery, direct measurements in the cell interior were nearly impossible because of the cell shape and the high number of MTs in this zone. The use of GFP-labeled proteins that specifically bind MT plus ends, such as EB1, made it possible to follow some parameters of MT dynamics (i. e. MT growing events) throughout the cell and was particularly useful for examining MT dynamics during mitosis [13, 14].

MT dynamic behavior can also be estimated by fluorescence recovery after photobleaching (FRAP) of YFP- or GFP-tubulin [15, 16]. This technique enables measurement of MT turnover, which reflects both dynamic instability and treadmilling. In addition, this technique appears to be very useful as MT dynamics seem to differ according to the proximity of MTs with the centrosome [17].

Regulation of microtubule dynamics in cells

Using the photobleaching strategy, Komarova et al. were able to image MT dynamics deep in the cytoplasm of CHO and NRK cells [17]. The majority of nascent MTs begin to grow from the centrosome without experiencing a shortening phase until they reach the cell cortex. In other words, dynamic instability is highly suppressed in the cell interior. This protection against depolymerization in the cell interior may be mediated by a kinesin-dependent pathway [18]. Several lines of evidence also implicate the actin cytoskeleton in stabilizing and guiding the MTs on their trek from the centrosome to the cell periphery [19, 20]. Indeed, dual-wavelength fluorescent speckle microscopy (FSM) [21] reveals growth of MTs along F-actin bundles in epithelial cells [22]. In addition, myosins sliding along actin filaments help in guiding MTs toward the actin-rich cortex [23].

In living cells, MT dynamics are regulated both spatially and temporally. In fact, MTs interact with an impressive number of binding proteins, and cellular MT dynamics are thus the result of the combined effect of stabilizing and destabilizing factors. Regulation can occur at many levels, some proteins regulating tubulin folding, some stabilizing existing MTs such as structural MAPs, and some interacting with MT ends to influence their dynamics, location and lifespan. The main MT-related proteins that regulate MT dynamics are presented in Table 1 and figure 1.

Table 1. Major microtubule-related proteins and their effects on MT dynamics

	MT-related proteins and homologues	Effects on MT dynamics	References
Structural MAPs	MAP1A; MAP1B; MAP2; MAP4; Tau	MT stabilization MT cross-linking suppression of MT dynamics	[39; 41]
Destabilizing +TIPs	kinesin-13 family	MT depolymerization catastrophe factors anti-rescue factors	[48; 49; 54]
Stabilizing +TIPs	EB1, 2, 3	MT stabilization MT elongation	[55; 62]
	Dis1/TOG family	MT polymerization anti-catastrophe factors	[57; 64; 65]
	CLIP family CLIP 170 ; CLIP 115	MT stabilization and capture at the cell cortex rescue factor	[58; 68; 69; 70]
	CLASPs	MT stabilization and capture at the cell cortex rescue factor suppression of MT dynamics	[58; 76]
	LIS1	anti-catastrophe factor	[60]
	dynactin	MT nucleation	[55]
	APC	MT polymerization MT stabilisation	[63]
	ACF7	actin cross-linking MT stabilization and capture at the cell cortex suppression of MT dynamics	[59]
Stathmin	stathmin	MT depolymerization catastrophe factor tubulin sequestering activity	[79]

Regulation by tubulin folding and isotype composition. Tubulin undergoes a complex folding and association process of one α and one β subunit. Monomer folding by the chaperonin CCT (chaperonin-containing TCP-1) and the formation of functional dimers by folding cofactors determine, together with transcriptional control, the amount of tubulin subunits available to polymerise which may influence MT dynamics [24].

In vertebrates, there are six α and seven β tubulin genes identified so far, leading to isotypes which mainly differ by their C-terminal sequences [25]. Tubulin isotypes are differently expressed depending on the stage of development and tissue. *In vitro*, β tubulin isotype composition strongly influence MT dynamics [3, 26, 27]. In addition, the C-terminal sequence, which differs among isotypes, is the major MAP binding site. Thus, isotype composition of MTs is involved in dynamics regulation. Some tubulin isotypes, such as class III β tubulin, were found to be overexpressed in tumors [28, 29]. This isotypic selection of tubulin in tumors may have important consequences for MT dynamics regulation and for the sensitivity or resistance to MTDs [29–34].

Structural MAPs. MT stability is promoted to a large degree by MAPs. One major family of MAPs, called structural MAPs, which includes MAP1A, MAP1B, MAP2, MAP4 and Tau, is responsible for cross-linking and stabilizing MTs in the cytosol (table 1 and fig. 1). MAP1A and MAP1B are large, filamentous molecules found mainly in axons and dendrites of neurons [35, 36]. MAP2, MAP4 and Tau are characterized by the presence of three or four repeats of an 18-residue sequence in their MT binding domain. MAP2 is found in dendrites, where it forms fibrous cross-bridges between MTs, and it also associates MTs with intermediate filaments. MAP2 expression has also been reported in non-neuronal tissues such as hair follicles [37]. MAP4, the most ubiquitous MAP, is found in neuronal and non-neuronal cells. Tau, which is much smaller than most other MAPs, is present in both axons and dendrites but also in other cell types, such as endothelial cells [unpublished observation] [38]. Dhamodharan and Wadsworth demonstrated that MAP2 and partially purified preparation of brain MAPs drastically suppress MT dynamic instability by decreasing the rate and extent of MT growth and shortening events, increasing the percentage of time spent in pause and decreasing the catastrophe frequency [39]. MAP2 is distributed inhomogeneously along MT length by forming high-density regions, which is important for its function as stops of depolymerization occur at these cluster sites [40].

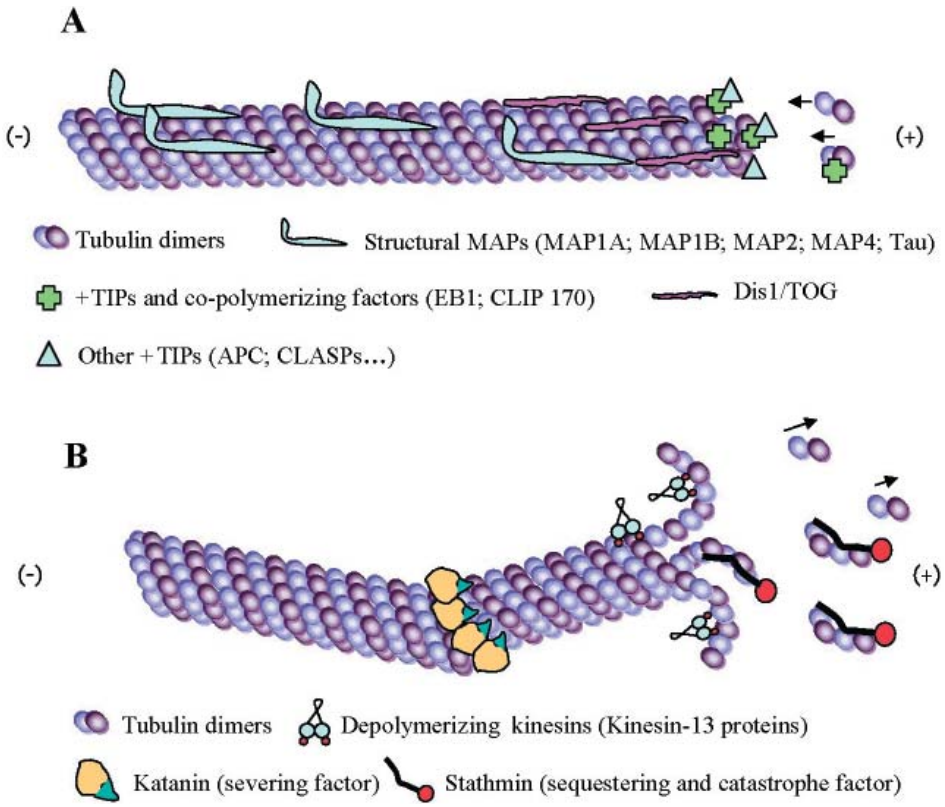

Figure 1. MT interactions with the major MT-related proteins. (*A*) A growing MT and various interactions with stabilizing factors. (*B*) A shortening MT and various interactions with destabilizing factors. (–) and (+) indicate the minus and the plus ends of the MT, respectively.

MAP effects on the dynamic instability parameters may, however, vary among the MAPs, and also among the different isoforms of a given MAP. For example, Tau protein exists in six isoforms derived from alternative splicing of a Tau messenger RNA (mRNA) and differs by the presence of either three- or four-repeat MT binding domains (3R and 4R, respectively). Bunker et al. demonstrated that 4R Tau reduced the rate and extent of both growth and shortening events in MCF7 cells expressing GFP tubulin, whereas 3R Tau was three-fold less potent than 4R Tau and had only a minimal ability to suppress shortening events [41]. This research group also analyzed the effects of 3R and 4R Tau proteins on MT growth rate *in vitro* [42]. First, the effects are dependent upon the tau:tubulin ratio. At low tau:tubulin ratio, 3R Tau isoforms reduced MT growth rate, whereas 4R Tau isoforms increased it. At high tau:tubulin ratio, both 4R and 3R Tau increased the growth rate. Altogether, these data show that the presence or absence of the exon 10-encoded sequence of Tau is important in the regulation of MT dynamics. Moreover, Tau is able to inhibit the destabilizing activity of XKCM1, a kinesin-13 family protein [43]. All these results illustrate the complexity of the regulation of MT dynamics by MAPs.

Expression, post-translational modifications and biological activities of various MAPs have been shown to be differentially regulated in tumor progression [44], probably resulting in differential regulation of MT dynamics. For example, Tau and MAP1B expression in a neuroblastoma cell line are increased upon differentiation [44] and MAP2 expression appears to be induced in primary cutaneous melanoma but absent in metastatic melanomas [45]. The consequences of these changes in MAP expression remain unclear. Indeed, during early tumor progression, MAP2 expression in melanoma inhibits cell division and metastasis [46] whereas a correlation between MAP2 expression and oral carcinogenesis was recently described [47].

Plus end binding proteins (+TIPs). A subset of MT binding proteins interacts specifically with MT plus ends. These proteins can be divided into MT destabilizing +TIPs, which belong to the kinesin-13 family [48, 49] and MT plus end tracking proteins, also termed stabilizing +TIPs (table 1).

Unlike most other kinesins, which are involved in MT-driven intracellular transport, kinesin-13 proteins concentrate at MT ends and can induce depolymerization *in vitro* [50] (fig. 1). These proteins influence MT dynamics in cells both during mitosis (e.g XKCM1) [51] and interphase [52, 53]. Mennella et *al.* evaluated the interphase functions, location and dynamics of two *Drosophila* kinesin-13 family members termed KLP10A and KLP59C [54]. In *Drosophila* S2 cells, both proteins contribute to MT depolymerization but affect distinct parameters of dynamic instability. KLP10A stimulates catastrophe, whereas KLP59C suppresses rescue. The authors also

demonstrated that KLP10A and KLP59C targets polymerizing and depolymerizing MT plus ends, respectively. Moreover, KLP10A is concentrated at the MT plus end by the plus end tracking protein EB1 [54].

MT plus end tracking proteins represent a highly diverse group of proteins, such as EB1, 2, 3; APC; Dis1/TOG; CLIP-170/CLIP-115; CLASP 1, 2; ACF7; Dynactin (p 150 Glued); LIS1; dynein [55–60] (fig. 1). Although these proteins can bind to MTs independently, evidence of interactions among them has led to the hypothesis of the existence of a plus end complex. By binding to the plus ends of MT, +TIPs influence MT structure and accessibility.

EB1 recruitment at MT plus ends plays an important role in the regulation of MT dynamics. Experiments with depletion or overexpression of EB1 in *Drosophila* S2 cells and frog egg extracts demonstrate that, during mitosis, EB1 localizes to elongating kinetochore MTs and stabilizes them, while in interphase it decreases pause duration [61, 62]. Moreover, EB1 interacts with dynactin, and overexpression of either of these two proteins induces MT bundles in cultured cells, suggesting that each may enhance MT stability. Dynactin has a potent MT nucleation effect, whereas EB1 has a potent elongation effect [55]. More recently, Wen et al. found that small amounts of EB1 are present at the tips of stable MTs (detyrosinated MTs) of fibroblasts where it forms a complex with APC and mDia [63].

The plus end binding Dis1/TOG family, which includes the human TOG, can bind directly to MTs and promote MT polymerization [64, 65]. Using purified TOG protein, Charrasse et al. showed that it increases the MT growth rate equally at both ends [65]. In this family, XMAP215, a frog homologue, suppresses MT catastrophe by opposing the activity of the destabilizing factor XKCM1, which belongs to the kinesin-13 family [66]. Studies in other systems, such as budding yeast, suggest that the antagonistic stabilizing/destabilizing effects of the Dis1/TOG family and depolymerizing kinesins have been conserved during evolution [67].

The proteins of the CLIP family, such as CLIP 170, are clearly involved in MT stabilization [68], acting as rescue factors [69, 70]. In addition, CLIP 170 and its homologues promote MT capture at cortical sites through direct interaction with dynein-dynactin [71–73] and the Rac1/Cdc42 effector IQGAP [74, 75]. The CLIP-associated proteins CLASP1 and CLASP2 stabilize MTs by promoting pauses and restricting MT growth and shortening events. They mediate interaction between the MT plus end and cell cortex and act as local rescue factors, probably through the formation of a complex with EB1 and MT tips [76]. The affinity of CLASPs for MTs is spatially regulated in cells [77]. The cooperation between CLIPs and CLASPs in the regulation of the MT network was recently reviewed [58].

Other +TIPs, such as ACF7, play a role similar to CLIP170 and CLASPs in the cortical capture and stabilization of MTs through interaction with the actin cytoskeleton [59].

Stathmin. The oncoprotein18 (op18)/stathmin belongs to a protein family involved in the regulation of MT dynamics in both interphase and mitosis [78]. Stathmin is a major MT-destabilizing phosphoprotein that promotes MT depolymerization by two distinct mechanisms [79] (table 1 and fig. 1). The first is a catastrophe-promoting activity involved in mitotic spindle regulation. The second is a tubulin-sequestering activity involved in the regulation of MT dynamics during interphase. Stathmin is inactivated by phosphorylation, which prevents its binding to tubulin [80]. Phosphorylation is mediated by a number of protein kinases, such as the Cdc2 kinase family [81, 82] and the small GTPases Rac and Cdc42 effectors, namely the p21-associated kinases (PAKs) [83, 84]. The activity of stathmin can also be regulated at the transcriptional level by p53 and E2F [85, 86]. Finally, stathmin has been reported to be overexpressed in various cancer malignancies, for example acute leukemia [87] and breast cancer [88]. Such stathmin overexpression can point to highly proliferative primary breast carcinomas [89], but the significance of this overexpression in cancer remains unclear.

Regulation by kinases and phosphatases. Phosphorylation has been shown to be a key regulatory postranslational modification of most of the MAPs described so far. In general, MAP phosphorylation leads to their detachment from the MT lattice and/or tubulin, thus decreasing MT stability. The phosphorylation state of these MAPs is thus crucial for the regulation of MT dynamics in cells and relies entirely on the concerted action of protein kinases and phosphatases.

Kinases inhibitors are known to suppress MT dynamic instability [90]. The most important kinases that regulate MT dynamics are those that control the cell cycle, such as the Cdc2 kinase family [91–93] and Cdk 5 in neurons [94], and those that control centromere localization, such as Aurora kinases [95]. PAKs that control cell migration [24, 83, 96], the mitogen-activated protein kinases (ERK, JNK) and GSK-3β, which are targets of extracellular signals involved in cell proliferation [97–99], are also invoved in the regulation of MT dynamics.

It is becoming increasingly evident that the functions of MT-regulating proteins are modulated both spatially and temporally by site-specific phosphorylation events. For example, stathmin phosphorylation by PAK1 inhibits its plus end catastrophe activity. However, *in vivo*, PAK1 activity is not sufficient to phosphorylate stathmin, indicating that additional pathways downstream of Rac 1 are required for stathmin regulation [100].

Phosphatases such as type 1 phosphatases (PP1) and type 2A phosphatases (PP2A), the major serine/threonine phosphatases in most cells [101], play a critical role in the regulation of MT dynamics in cells, as shown by the increase in MT dynamics induced by okadaic acid treatment [90]. Notably, PP2As are required to maintain the short steady-state length of MTs in mitosis by modulating catastrophe frequency through stathmin regulation. In contrast, PP1s are only required to control MT dynamics during transitions into and out of mitosis [102]. Interestingly, some specific PP2A isoforms are regulated by their binding to MTs [103, 104]. MT anchoring of PP2A may serve to sequester selective intracellular pools of enzymes in an inactive state, and to promote kinase-mediated phosphorylation of MAPs. MT depolymerization results in the release and activation of PP2A, and the dephosphorylation of MAPs such as Tau [105, 106].
Finally, it is of crucial importance to identify the protein kinases and phosphatases that regulate MAP phosphorylation *in vivo* as well as the signaling cascades that regulate them. Overall MT dynamics may result from a balance between the individual effects of these regulatory proteins.

Cellular functions depending on microtubule dynamics

Among the various cell functions regulated by MT dynamics, the most important ones that are involved in tumor progression are the control of cell proliferation and migration. Besides being powerful anticancer agents, MTDs that either depolymerize the MT network, such as vinca alkaloids, or stabilize it, such as taxanes, are valuable tools for deciphering the roles of MT dynamics and the importance of their regulation in cellular contexts.

Mitosis and cell proliferation

In eukaryotes, the mitotic spindle is composed of MTs extending from two opposing spindle poles: the minus ends, anchored to the poles, and the plus ends, extending away from them. Three distinct subsets of mitotic MTs can be characterized: kinetochore MTs (kMTs), whose plus ends attach to sister chromatids at the kinetochore, interpolar or pole-to-pole MTs, which overlap MTs from the opposite pole at the spindle midzone, and astral MTs, extending away from the spindle (fig. 2A). Direct analysis of MT dynamics and measurements of the dynamic instability parameters are hard to achieve during mitosis, due to the brief time window available and to image resolution problems at the central spindle. However, in spite of the significant complexity of this process, the intimate mechanisms of mitotic spindle dynamics and their regulation are increasingly being investigated. For a recent review, see Kline-Smith and Walczac [107].
Mathematical and computer modeling [108] demonstrate that the cell predominantly regulates MT transition frequencies in order to reorganize the interphase array into a mitotic spindle. MT turnover is much higher in mitotic cells than in interphase cells [109, 110], and this is thought to be responsible for mitotic spindle establishment and its maintenance. During early prophase, it has been shown that MT nucleation at the centrosomes increases 4-fold [14]. The growth of these MTs is stabilized in the vicinity of the nuclear envelope [13]. MTs from the two developing poles can interact with each other to form overlapping associations (fig. 2A). Thus, formation of two MT subsets, interpolar and astral, starts prior to nuclear envelope breakdown (NEB). When the nuclear envelope is still intact, non-nuclear MT dynamics regulators are predicted to be responsible for early events involved in spindle assembly.
Upon NEB, as nuclear factors and mitotic chromosomes enter the cytoplasm, MT dynamics increase dramatically; this is the hallmark of the 'Search-and-Capture' model of spindle assembly. Indeed, during prometaphase, spindle MT plus ends probe the cytoplasm until linkage with a chromosomal kinetochore is established. Kinetochores, selectively stabilizing their associated MTs, enable the formation of mature and stable K fibers containing approximately 25 MTs in vertebrate metaphase cells [111, 112]. If dynamic instability is sufficient to explain chro-

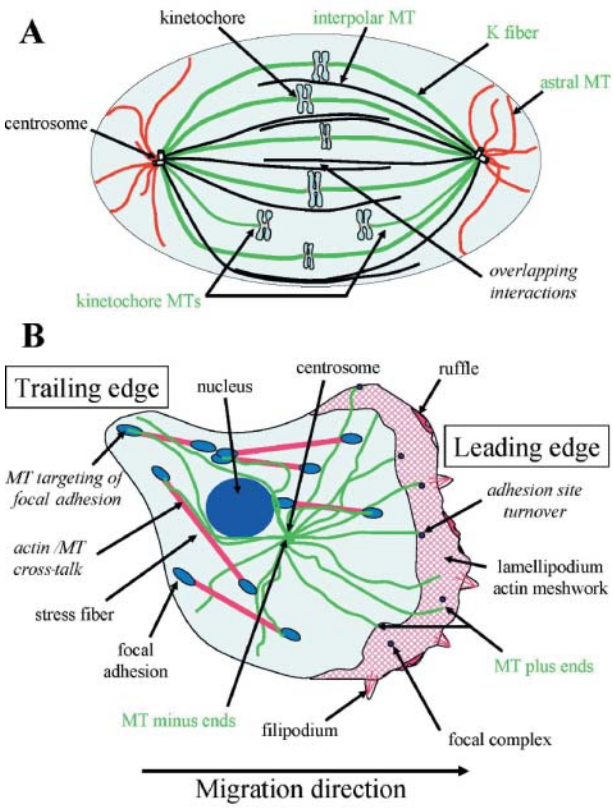

Figure 2. Structural organization of the MT network in mitotic cell at the prometaphase stage (*A*) and in migrating cell (*B*).

mosome capture within mitotic timescales, mathematical modeling predicts that MT dynamics may be biased toward the chromosomes by the presence of a spatial gradient of a 'stabilizing' factor (e.g RanGTP gradient) to enable an accurate congression of chromosomes [113].

The availability and distribution of MT plus ends is a source of differential stability within the spindle. The majority of kMTs are continuous from the centrosome to the kinetochore [114]. In contrast, astral and interpolar MTs have many free plus ends that can be affected by the balance of cytoplasmic MT-regulating proteins (see above). Overall dynamicity of astral MT plus ends, in prometaphase or metaphase epithelial cells, is increased 4-fold as compared with interphase MTs [115]. This increase is mainly due to a decrease in rescue frequency and an increase in catastrophe frequency. In addition, the time spent in pause is dramatically decreased. In these experiments, no significant change in MT growth and shortening rates was observed. In contrast, Piehl and Cassimeris [13] demonstrated, by following EB1-GFP movement in living epithelial cells, a 1.5-fold increase in MT growth rate during prophase, prometaphase and metaphase, as compared with interphase. Elsewhere, MT growth rate does not seem to be spatially regulated during mitosis, as seen when comparing astral and spindle MTs in prometaphase and metaphase [13]. As combinations of regulatory factors are active during different phases of the cell cycle, they may be responsible for the similar MT growth and shortening rates observed throughout the cell cycle.

A subset of more stable astral MTs has been described in metaphase [115]. Interactions of their plus ends with the cell cortex might contribute to spindle positioning. Moreover, several events have been proposed to stabilize MT minus ends at poles during prometaphase: NuMA and TPX2 transport to spindle poles [116–118], increased levels of γ tubulin [14]; localized activation of MAPs; and activation of MT assembly promoters by small GTPase Ran [119]. Many proteins affect chromosome alignment: the kinetochore-associated kinesin CENP-E is required for stability of kinetochore-MT attachment [120], NuMA organization of poles is required for proper chromosome movement [117, 118] and centromere-associated MCAK is required for resolving improper kinetochore attachments that can disrupt chromosome congression and alignment [121–123]. Despite their overall increased stability, kMT plus ends exhibit highly regulated dynamics that are associated with chromosome movement in somatic cells [112, 124]. Chromosome-associated motors, called chromokinesins, may participate to force production at chromosome arms, helping chromosome alignment and orientation. Although chromokinesins and kinetochore-associated proteins certainly play a role in chromosome alignment, the detailed regulation of MT dynamics during congression is poorly understood, partly due to the difficulties of imaging the highly varied movements of spindle MTs and chromosomes during prometaphase. Elsewhere, accurate regulation of MT dynamics prevents, at least in part, chromosome segregation defaults. For example, MT dynamics can be differentially regulated to counteract merotelical orientation of chromosomes [125].

During anaphase, two mechanisms of K fiber depolymerization exist to drive chromosome segregation. In somatic cells, kMT plus end depolymerization dominates, while treadmilling appears to play a minor role in force production for chromosome segregation. In parallel, overlapping MTs at the spindle midzone polymerize at their plus ends. These overlapping interactions participate in spindle elongation and pole removal, ultimately leading to cytokinesis. A recent study on metaphase-arrested budding yeast cells demonstrated that separase activation at anaphase onset, which triggers chromosome segregation through cohesin cleavage, induces MT stabilization, resulting in a 60% decrease in spindle MT turnover [126]. Separase also activates the phosphatase Cdc14, which is responsible for MT dynamics silencing. Altogether, these data show that MT dynamics are highly regulated during cell division by a combination of MT-regulating proteins, nuclear factors and cell cycle-related proteins.

Cell migration

Cell migration is an essential process for all multicellular organisms. It is necessary for many physiological processes not only during development but also throughout life, including wound repair and immune surveillance. It is also involved in pathological processes such as tumor neoangiogenesis and metastasis. Directional cell migration is usually initiated in response to extracellular stimuli. A migrating cell is highly polarized, as shown by the asymmetrical distribution of signaling molecules, cytoskeleton, centrosome, golgi apparatus and directed membrane trafficking (fig. 2B).

Migration can be viewed as a periodically repeating sequence of events that includes formation of pseudopodial protrusions, attachment and translocation of the cell body in the direction of the new adhesion sites [127]. All these events can, theoretically, be served by the actin cytoskeleton, in the absence of MTs [128]. However, the pioneering work of Vasiliev [129] showed that a majority of cell types does require MTs for directional locomotion.

MT dynamic instability is important to generate an asymmetrical MT array and maintain cell shape [130–132]. In most undifferentiated and non-polarized mammalian cells, MT arrays are arranged radially and are composed of highly dynamic MTs. In many migrating cells, MT arrays become polarized by selective formation of a subset of unusually stable MTs at the leading edge [133–138]. This specific stabilization of MT plus ends is mediated

by EB1/APC [63] and by integrin- and focal adhesion kinase (FAK)-facilitated Rho-mDia signaling pathways [138]. These stable MTs have a long half-life and are capped at their plus ends [139]. They accumulate post-translationally modified tubulin, such as detyrosinated tubulin, in which the C-terminal tyrosine of the α subunit is removed by tubulin carboxy peptidase [140]. Stabilized MTs may function as specialized tracks for vesicle and cytoskeletal trafficking. They also enable centrosome reorientation toward the leading edge. This centrosome reorientation is cell-type specific and is not required for directed cell migration [141]. It is becoming evident that MTs cannot act solely as tracks for directed delivery of motility-required components to the leading edge but that MT dynamics regulate cell migration.

The dependence on MTs for the locomotion of a particular cell type correlates with the presence of focal adhesions. Cells that form focal complex-type adhesions but do not convert them into focal adhesions can move independently of MTs. In contrast, cells that require MTs for migration are well-attached cells that demonstrate typical focal adhesions in addition to focal complexes [142]. On the one hand, MT depolymerization in such cells leads to a further increase in the number and size of focal adhesions [143–145], which impedes cell migration by increasing their adhesion to a level incompatible with locomotion [146]. Such processes arise through the activation of Rho GTPases [143–145]. On the other hand, recovery from treatment with an MT depolymerizing agent leads to MT regrowth toward and into the membrane ruffle and to activation of Rac1 [147]. It has been suggested that MT outgrowth activates Rac1 at the cell front to promote migration. Because Rac1 promotes MT growth through its effectors [100, 148, 149] and because MT growth stimulates the activation of Rac1 [147], MTs and Rac1 seem to be part of a feedback loop that maintains the activity of Rac1 for cell migration. Thus, a direct correlation exists between specific phases of MT dynamics and the activity of the Rho GTPase proteins that direct actin organization and substrate adhesion dynamics. MT dynamics might modulate the activity of Rho family GTPases by regulating GEFs such as RhoGEF-H1 [150].

Multiple targeting events of focal adhesion by MT plus ends induce their dissociation and release from the substrate to promote cell migration [20, 151, 152]. This regulation of adhesion site turnover appears to be dependent upon kinesin-1 [153], dynamin and FAK but independent of Rho and Rac activity [154]. The effect of MTs on cell migration might be imputed to their ability to regulate the turnover of adhesions, to enable protrusion and lamellipodium formation at the cell front, and to promote retraction at the rear of cells.

Even if MT involvement in cell migration is now evident, the relationship between the parameters of individual MT dynamic instability and cell migration remains poorly described. Cell locomotion and lamellipodial size have been examined in migrating cells [155]. Faster cell movement and a larger lamellipodial area have been found to be correlated with MTs spending more time growing, a greater number of MT ends near the base of lamellipodia, and faster MT shortening rates. Slower movement and smaller lamellipodial area are associated with MTs spending more time in pause.

MT plus ends are regulated differently in the different subcellular regions of a migrating cell. At the leading edge, MTs tend to persist in growth, whereas in the cell body they spend more time pausing and shortening, and the catastrophe frequency is higher [156]. More recently, the same research group analysed MT dynamics in migrating CHO and LLCPK cells at both the leading and the trailing edges [157]. MT dynamicity was 2- to 13-fold higher at the trailing edge than at the leading edge. Interestingly, no difference between MT growing rates at the leading edge and the trailing edge was observed. Nevertheless, MTs at the leading edge experienced a lower shortening rate, a lower catastrophe frequency and a higher duration of pauses as compared with the trailing edge. Thus, in migrating cells, a gradient of MT dynamic instability occurs from the leading edge to the trailing edge, and it appears to be crucial for cell polarity maintenance and the motility process.

MT dynamics and cell signaling

Connections between the MT cytoskeleton and cell signaling remain complex and poorly understood. Intracellular signaling, as from Rho GTPases and MAPKs (mitogen-activated protein kinases), regulates many cellular processes, including MT network functions [158–164]. A pool of survivin, an apoptosis inhibitor (IAP), associates with MTs and participates in mitotic spindle function [165, 166]. Forced expression of survivin in epitheloid carcinoma cells profoundly influences MT dynamics, with reduction of pole-to-pole distance at metaphase and stabilization of MTs against nocodazole-induced depolymerization [167]. Inhibition of PI3K (phosphatidylinositol 3-kinase)/Akt and MAPK pathways diminishes survivin induction and sensitizes cells to taxane-mediated cell death [168].

Conversely, MT dynamics may influence cell signaling. Various proteins are associated with MTs, and modulation of MT dynamics is likely to affect their sequestration and/or activation. MTDs such as vinca alkaloids, nocodazole and paclitaxel activate the nuclear factor kB (NF-κB) signaling pathway through the degradation of the NF-κB inhibitor (IκB), located on intact MTs [169–171]. Elsewhere, about 40% of total MAPKs are linked to MTs [172] via kinesin-related motor molecules [173]. Similarly, MTs probably regulate Rho GTPase activity by sequestering GEFs such as GEF-H1 [150]. Bim, a proap-

optotic factor of the Bcl-2 family, is also associated with MTs. It represents an important link between the MT network and the apoptotic machinery, as lymphocytes *bim –/–* are refractory to MT perturbations [174]. Upon disruption of MT network functions by MTDs, freed Bim translocates to the mitochondria where it constitutes an initiating event in apoptotic signaling [175, 176]. In response to DNA damage, the tumor suppressor protein p53 is transported to the nucleus via the interphase MT minus end-directed motor dynein [176–179]. Thus, the nuclear p53 activity depends on MT integrity [178–181]. These results suggest that MT dynamics also regulate intracellular transport in addition to motor proteins.

In eukaryotic cells, membrane-bounded vesicles, organelles such as mitochondria, RNA and proteins are frequently transported many micrometers along well-defined routes in the cytosol and delivered to particular addresses. The importance of MT-directed transport has been largely investigated in neuronal biology [182]. It is important for information transduction, such as MT-motor dependent transduction of neurotrophic signals. The basic components of the MT-dependent transport system are MT motor proteins, kinesin and dynein, and the polarized MT tracks along which they carry cargoes. Nearly all kinesins move cargo toward the MT plus ends (anterograde transport), whereas dyneins transport cargo toward the MT minus ends (retrograde transport) [183]. The net direction of transport depends on the balance between plus end-directed and minus end-directed motors. Several kinesins have been reported to alter MT dynamics, including kinesin-13 family proteins (e. g. MCAK), which trigger MT depolymerization (see above). Moreover, MAPs, which regulate MT dynamics, can profoundly affect patterns of motor activity [184]. For example, MAP2 can inhibit kinesin-driven MT sliding [185]. Most cytoplasmic MTs have a substantial complement of MT-related proteins, and their regulation, which in turn regulates MT dynamics, could thus profoundly modify patterns of MT-based intracellular transport.

Microtubule dynamics as a therapeutic target in oncology

The critical role that MTs play in cell division makes them a very suitable target for the development of chemotherapeutic drugs against rapidly dividing tumor cells such as leukemias and lymphomas. In addition, these coumpounds are also broadly active against more slow growing tumors, such as some breast, lung and ovary cancers. MTs are thus the target for a large and chemically diverse group of molecules called MTDs. The effectiveness of MTDs has been validated by the successful use of several vinca alkaloids and taxanes for the treatment of a wide variety of human cancers. Their clinical success has prompted a worldwide search for new compounds with improved characteristics (solubility, pharmacokinetics, oral route administration, non-Pgp-dependent and efficiency in tumor-resistant cells).

Suppression of microtubule dynamics disturbs mitotic progression

MTDs exert their inhibitory effects on cancer cell proliferation primarily by blocking mitosis, which requires a finely regulated control of MT dynamics. MTDs are therefore referred as anti-mitotic drugs. They are usually divided into two groups when they are used at relatively high concentrations. MT-depolymerizing agents such as the vinca alkaloids, colchicinoids and estramustine inhibit MT polymerization, depolymerize MTs and decrease the MT polymer mass. In contrast, MT stabilizing agents, such as taxanes, promote MT polymerization, stabilize MTs and increase MT polymer mass in cells [186].

More recently, it has been proved that MTDs, at low but clinically relevant concentrations, potently suppress MT dynamics without affecting MT polymer mass [186–188]. This occurs through several ways, depending on cell types, on the tubulin binding site (i. e. colchicine, vinca alkaloid or paclitaxel binding site) but also on molecules [187, 189, 190]. Therefore, MTDs can suppress MT dynamics either by decreasing growth and shortening rates, or by affecting transition frequencies or even both [11, 186]. However, these diverse alterations of MT dynamics lead to very similar mitotic spindle abnormalities and usually result in mitotic block (fig. 3) [187, 189, 190]. The effects of MTDs on MT dynamics in tumor cells were measured in interphase cells. The question arises whether these measurements also reflect drug effects on the mitotic spindle. Indirect evidences suggest that MTDs also suppress spindle MT dynamics. First, the level of MT dynamics suppression by MTDs in interphasic cells correlates with the level of mitotic block in the cell population [187, 188, 191]. In addition, studies on the dynamics of centromeres, kinetochores and their attached spindle MTs in human osteosarcoma cells indicate that mitotic block is closely associated with suppression of centromere dynamics by paclitaxel and vinca alkaloids [192, 193]. Thus, it is reasonable to argue that the anti-mitotic and anti-cancer activity of MTDs is largely due to the suppression of spindle MT dynamics, instead of their effects on the MT polymer mass, as previously thought. The anti-tumor actions of MTDs appear to involve their capacity of MT dynamics suppression to levels below those necessary for MTs to achieve their mitotic functions, especially the transition from metaphase to anaphase.

Interestingly, a low suppression of MT dynamic instability by MTDs, which does not allow the accumulation of cells in mitosis, still inhibits cell proliferation and induces apoptosis in tumor cells [190]. This effect cor-

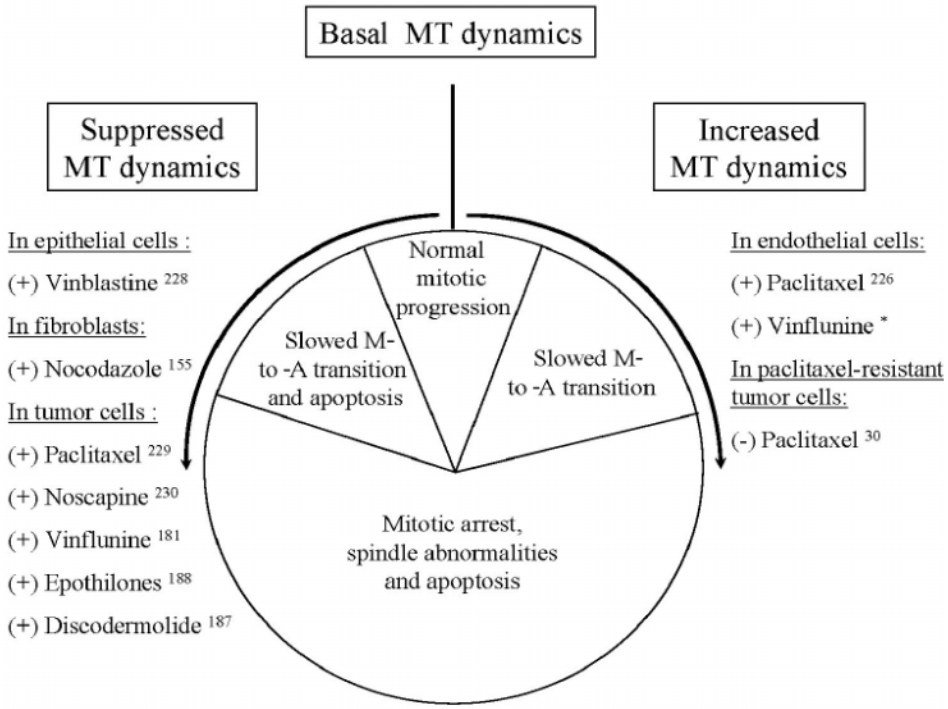

Figure 3. Consequences of MT dynamics modulation by MT-targeted drugs on mitotic progression. M-to-A transition = metaphase-to-anaphase transition. *, unpublished observation.

relates with a slowing of the mitotic progression at the metaphase-to-anaphase transition and subsequent mitotic slippage [187, 194, 195]. During prometaphase, the plus ends of dynamic spindle MTs appear to probe the cytoplasm until linkage with a chromosomal kinetochore is established. Reduced MT dynamics in cells treated with MTDs may result in impaired probing of the intracellular space by MTs and to the attachment of a fewer number of MTs to kinetochores, thus delaying chromosome congression to the metaphase plate and also reducing the tension necessary for the metaphase-to-anaphase transition [192, 193, 196, 197]. Slowing of metaphase-to-anaphase transition usually lead to chromosome alignment defaults and a correlation between MT dynamics suppression and mitotic spindle abnormalities has been demonstrated [187].

Interestingly, A549–T12 and A549-T24 tumor cells, which are 9- and 17- fold resistant to paclitaxel, respectively, as compared with parental A549 cells, require low concentrations of paclitaxel for proliferation. In these cells, in the absence of added paclitaxel MT dynamic instability was drastically increased proportional to the resistance level [30]. Thus, the decreased paclitaxel sensitivity of these cells can be explained by their increased MT dynamics. More drug molecules are thus needed to suppress MT dynamics below the levels necessary to achieve their functions. Moreover, when grown without paclitaxel, A549-T12 cells were blocked at the metaphase/anaphase transition and displayed abnormal mitotic spindles with uncongressed chromosomes [30]. When grown with low paclitaxel concentrations, normal mitotic spindle is recovered, probably because MT dynamics were slowed to a range compatible with their functions. These results suggest that both excessively rapid dynamics as well as suppressed dynamics correlate with impaired mitotic spindle function and inhibition of cell proliferation (fig. 3).

Suppression of MT dynamics induces apoptosis

It has been clearly demonstrated that MTDs induce apoptosis mainly through the intrinsic pathway, as reviewed by Carre and Braguer [198]. Apoptosis induction correlates with the suppression level of MT dynamics [191] through a mechanism which remains unclear. Interestingly, suppression of MT dynamics and alteration of mitochondrial parameters are two early and concomitant events. These early mitochondrial disturbances (i.e. reactive oxygen species [ROS] production, decrease in mitochondrial respiration rate, alteration of mitochondrial membrane potential) [199, 200] finally lead to the release of proapoptotic factors into the cytosol and ultimatly caspase activation [201]. These MTD effects may be mediated by the presence of a pool of mitochondrial tubulin [202]. However, there is still no link between MT dynamics suppression and the mitochondrial apoptotic pathway.

Bim, a proapoptotic member of the Bcl-2 family, is sequestered by MTs and released upon MT network disruption (see above). In addition, it has been shown that p53 can translocate to the nucleus and also to the mitochondria, following suppression of MT dynamics by MTDs [178, 179, 181]. Elsewhere, C19ORF5, a sequence homologue of MAP1A and MAP1B, selectively

associates with MTs stabilized by paclitaxel or by the MT stabilizer RASSF1A, and it also binds to mitochondria-associated proteins [203, 204]. Thus, MT-regulating proteins as well as proteins that are sequestered or transported by MTs may represent an important link between MT dynamics suppression and apoptosis induction.

MT dynamics suppression inhibits cell migration

One of the main causes of failure in the treatment of patients with solid malignancies is the difficulty in controlling or preventing the spread of metastases. The process of tumor cell invasion and metastasis is conventionally understood as the migration of individual cells that detach from the primary tumor, enter lymphatic vessels or the bloodstream, and seed in distant organs.

The effects of MTDs on cell migration and invasion remain very controversial, probably due to different tumor cell line behaviors, different culture conditions, but most important to different drug concentrations [205–211]. Both paclitaxel and nocodazole, at concentrations that significantly suppress MT dynamics without modifying the MT polymer mass, reduced up to 60% the rate of locomotion of NRK fibroblasts [155, 209]. In these cells, inhibition of cell migration was correlated with MT dynamics suppression, limiting the number of MT plus ends that effectively reach the lamillipodium base to regulate its formation. Interestingly, Belotti et al. demonstrated that the anti-migratory effect of paclitaxel occurs at non-anti-proliferative concentrations in tumor cells [212].

The paucity of studies that effectively link MT dynamics and cell migration highlights the need of further experiments to decipher the exact role of MT dynamics in tumor cell migration, invasion and metastasis.

Targeting MT dynamics in endothelial cells

The tumor vasculature currently represents a new target for the development of cancer therapy, since functional vasculature is critical for both primary tumor growth and metastatic invasion [213, 214]. The vasculature is easily accessible to therapeutic agents. Two approaches are used to target the vascular functions in tumors: antivascular therapy, which aims at a rapid and extensive shutdown of the tumor vasculature, leading to tumor cell death [215, 216], and antiangiogenic therapy, which consists in an inhibition of new blood vessel formation.

Antivascular therapy. Historically, the MT-depolymerizing drug colchicine was the first MTD to show antivascular effects [217, 218]. Unfortunately, the therapeutic window was very limited due to the drug toxicity. Vinblastine and vincristine also induce vasculature damage in animal tumors but at concentrations close to the maximum tolerated doses [219]. Nevertheless, the ability of several novel MTDs (i.e. combretastatine, vinflunine and ZD 6126) to rapidly shut down existing tumor vasculature, at doses well below the maximum tolerated doses, was recently demonstrated [216, 220]. Interestingly, these vascular targeting agents seem to damage tumor vasculature without significantly altering normal tissues [221]. The reason for this specificity is still unknown, but it was imputated to differences in the architecture, and cellular and biochemical composition between normal and tumor blood vessels [222, 223]. Finally, the anti-vascular effect of the MTDs relies on MT network disruption in tumor-derived endothelial cells rather than on MT dynamics alteration.

Antiangiogenic therapy. In contrast to the antivascular approach, the antiangiogenic approach is likely to be related to MTD effects on MT dynamics in endothelial cells. MTDs were among the first anti-cancer agents reported to have antiangiogenic properties at relatively low concentrations, as compared with those used for antitumor effects [212, 224]. Neo-angiogenesis is the formation of new blood vessels from the pre-existing vasculature toward and into tumors. This complex and dynamic process requires activation, proliferation, migration and differentiation of endothelial cells. Inhibition of one or more of these steps may dramatically alter neo-angiogenesis *in vivo*. Until recently, the majority of the research in this area focused on the antiproliferating impact of antiangiogenic therapy. Paclitaxel, at concentrations > 10 nM, inhibits endothelial cell proliferation through mitotic block and the mitochondrial apoptotic pathway [225]. This cytotoxic effect of paclitaxel classically correlates with MT dynamics suppression as observed in tumor cells [226]. In contrast, below 10 nM, paclitaxel inhibits endothelial cell proliferation without any accompanying mitotic arrest or apoptosis. This cytostatic effect correlates with a slowing of the metaphase-to-anaphase transition [225]. Unexpectedly, these cytostatic concentrations of paclitaxel cause a strong increase in MT dynamic instability in living endothelial cells (fig. 3) [226]. Increased MT dynamic instability correlates with angiogenesis inhibition, as measured by the inhibition of capillary-like tube formation on Matrigel. The antiangiogenic effect of paclitaxel also occurs at concentrations that do not inhibit endothelial proliferation. Elsewhere, it has been shown that taxanes can inhibit endothelial cell migration, probably through the impairement of centrosome reorientation toward the direction of migration [227]. Increased MT dynamics and antiangiogenesis at non-anti-proliferative concentrations have also been demonstrated for vinflunine, the newest vinca alkaloid in development [unpublished observation]. Interestingly, concentrations of vinflunine that increased MT dynamics in living endothelial cells also inhibited endothelial cell migration (unpublished observation). These results suggest that the increase in MT dynamics is a common feature of MTD, at non-cytotoxic concentrations and seems

to be specific to endothelial cells, as it is not observed in cancer cells [226]. Further studies are needed to better understand the mechanism by which MTDs increase MT dynamics in these cells, and the molecular link between the increase in MT dynamics and the antiangiogenic properties of MTDs.

Conclusion

MT dynamics allow cell adaptation to rapid changes in their microenvironment. MT dynamics regulation by intracellular components, which directly interact with MTs, is beginning to be elucidated. However, little is known about how extra-cellular stimuli regulate the MT network. Development of potent new tools such as genomics and proteomics will improve the identification of proteins regulating the MT network. It will also lead to a better understanding of tumor progression regulators. Finally, identification of highly specific targets of MT dynamics will be very helpful for clinical research to improve cancer therapy.

Acknowledgements. We thank Drs. M.-A. Jordan and L. Wilson for invaluable help in understanding microtubule dynamics and for critical and fruitful discussions. We gratefully acknowledge Dr. K. Kamath and our laboratory co-workers who contributed to this work and to helpful discussions. We thank the Groupement des Entreprises Françaises dans la Lutte contre le Cancer (Marseille, Provence), the Association pour la Recherche sur le Cancer and the Assistance Publique-Hôpitaux de Marseille for grant support.

1 Mitchison T. and Kirschner M. (1984) Dynamic instability of microtubule growth. Nature **312:** 237–242
2 Mitchison T. and Kirschner M. (1984) Microtubule assembly nucleated by isolated centrosomes. Nature **312:** 232–237
3 Panda D., Miller H. P., Banerjee A., Luduena R. F. and Wilson L. (1994) Microtubule dynamics in vitro are regulated by the tubulin isotype composition. Proc. Natl. Acad. Sci. USA **91:** 11358–11362
4 Billger M. A., Bhattacharjee G. and Williams R. C. Jr. (1996) Dynamic instability of microtubules assembled from microtubule-associated protein-free tubulin: neither variability of growth and shortening rates nor "rescue" requires microtubule-associated proteins. Biochemistry **35:** 13656–13663
5 Joshi H. C. (1998) Microtubule dynamics in living cells. Curr. Opin. Cell Biol. **10:** 35–44
6 Chretien D., Jainosi I., Taveau J. C. and Flyvbjerg H. (1999) Microtubule's conformational cap. Cell. Struct. Funct. **24:** 299–303
7 Panda D., Miller H. P. and Wilson L. (2002) Determination of the size and chemical nature of the stabilizing "cap" at microtubule ends using modulators of polymerization dynamics. Biochemistry **41:** 1609–1617
8 Margolis R. L. and Wilson L. (1998) Microtubule treadmilling: what goes around comes around. Bioessays **20:** 830–836
9 Waterman-Storer C. M. and Salmon E. D. (1997) Microtubule dynamics: treadmilling comes around again. Curr. Biol. **7:** R369–372
10 Howard J. and Hyman A. A. (2003) Dynamics and mechanics of the microtubule plus end. Nature **422:** 753–758
11 Jordan M. A. and Wilson L. (2004) Microtubules as a target for anticancer drugs. Nat. Rev. Cancer **4:** 253–265
12 Toso R. J., Jordan M. A., Farrell K. W., Matsumoto B. and Wilson L. (1993) Kinetic stabilization of microtubule dynamic instability in vitro by vinblastine. Biochemistry **32:** 1285–1293
13 Piehl M. and Cassimeris L. (2003) Organization and dynamics of growing microtubule plus ends during early mitosis. Mol. Biol. Cell **14:** 916–925
14 Piehl M., Tulu U. S., Wadsworth P. and Cassimeris L. (2004) Centrosome maturation: measurement of microtubule nucleation throughout the cell cycle by using GFP-tagged EB1. Proc. Natl. Acad. Sci. USA **101:** 1584–1588
15 Shaw S. L., Kamyar R. and Ehrhardt D. W. (2003) Sustained microtubule treadmilling in Arabidopsis cortical arrays. Science **300:** 1715–1718
16 Lolkema M. P., Mehra N., Jorna A. S., van Beest M., Giles R. H. and Voest E. E. (2004) The von Hippel-Lindau tumor suppressor protein influences microtubule dynamics at the cell periphery. Exp. Cell Res. **301:** 139–146
17 Komarova Y. A., Vorobjev I. A. and Borisy G. G. (2002) Life cycle of MTs: persistent growth in the cell interior, asymmetric transition frequencies and effects of the cell boundary. J. Cell Sci. **115:** 3527–3539
18 Marceiller J., Drechou A., Durand G., Perez F. and Pous C. (2005) Kinesin is involved in protecting nascent microtubules from disassembly after recovery from nocodazole treatment. Exp. Cell Res. **304:** 483–492
19 Goode B. L., Drubin D. G. and Barnes G. (2000) Functional cooperation between the microtubule and actin cytoskeletons. Curr. Opin. Cell Biol. **12:** 63–71
20 Small J. V. and Kaverina I. (2003) Microtubules meet substrate adhesions to arrange cell polarity. Curr. Opin. Cell Biol. **15:** 40–47
21 Waterman-Storer C. M. and Salmon E. D. (1997) 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. **139:** 417–434
22 Salmon W. C., Adams M. C. and Waterman-Storer C. M. (2002) Dual-wavelength fluorescent speckle microscopy reveals coupling of microtubule and actin movements in migrating cells. J. Cell Biol. **158:** 31–37
23 Rodriguez O. C., Schaefer A. W., Mandato C. A., Forscher P., Bement W. M. and Waterman-Storer C. M. (2003) Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat. Cell Biol. **5:** 599–609
24 Vadlamudi R. K., Barnes C. J., Rayala S., Li F., Balasenthil S., Marcus S. et al. (2005) p21-activated kinase 1 regulates microtubule dynamics by phosphorylating tubulin cofactor B. Mol. Cell. Biol. **25:** 3726–3736
25 Luduena R. F. (1998) Multiple forms of tubulin: different gene products and covalent modifications. Int. Rev. Cytol. **178:** 207–275
26 Banerjee A., Roach M. C., Trcka P. and Luduena R. F. (1990) Increased microtubule assembly in bovine brain tubulin lacking the type III isotype of beta-tubulin. J. Biol. Chem. **265:** 1794–1799
27 Lu Q. and Luduena R. F. (1994) In vitro analysis of microtubule assembly of isotypically pure tubulin dimers. Intrinsic differences in the assembly properties of alpha beta II, alpha beta III and alpha beta IV tubulin dimers in the absence of microtubule-associated proteins. J. Biol. Chem. **269:** 2041–2047
28 Mozzetti S., Ferlini C., Concolino P., Filippetti F., Raspaglio G., Prislei S. et al. (2005) Class III beta-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients. Clin. Cancer Res. **11:** 298–305
29 Seve P. and Dumontet C. (2005) Chemoresistance in non-small cell lung cancer. Curr. Med. Chem. Anti-Canc. Agents **5:** 73–88
30 Goncalves A., Braguer D., Kamath K., Martello L., Briand C., Horwitz S. et al. (2001) Resistance to Taxol in lung cancer cells

associated with increased microtubule dynamics. Proc. Natl. Acad. Sci. USA **98:** 11737–11742
31 Verdier-Pinard P., Wang F., Martello L., Burd B., Orr G. A. and Horwitz S. B. (2003) Analysis of tubulin isotypes and mutations from taxol-resistant cells by combined isoelectrofocusing and mass spectrometry. Biochemistry **42:** 5349–5357
32 Kamath K., Wilson L., Cabral F. and Jordan M. A. (2005) BetaIII-tubulin induces paclitaxel resistance in association with reduced effects on microtubule dynamic instability. J. Biol. Chem. **280:** 12902–12907
33 Shalli K., Brown I., Heys S. D. and Schofield A. C. (2005) Alterations of beta-tubulin isotypes in breast cancer cells resistant to docetaxel. FASEB J. **19:** 1299–1301
34 Ferguson R. E., Taylor C. , Stanley A., Butler E., Joyce A., Harnden P. et al. (2005) Resistance to the tubulin-binding agents in renal cell carcinoma: no mutations in the class I beta-tubulin gene but changes in tubulin isotype protein expression. Clin. Cancer Res. **11:** 3439–3445
35 Pedrotti B., Ulloa L., Avila J. and Islam K. (1996) Characterization of microtubule-associated protein MAP1B: phosphorylation state, light chains and binding to microtubules. Biochemistry **35:** 3016–3023
36 Chien C. L., Lu K. S., Lin Y. S., Hsieh C. J. and Hirokawa N. (2005) The functional cooperation of MAP1A heavy chain and light chain 2 in the binding of microtubules. Exp. Cell Res. **308:** 446–458
37 Hallman J. R., Fang D., Setaluri V. and White W. L. (2002) Microtubule associated protein (MAP-2) expression defines the companion layer of the anagen hair follicle and an analogous zone in the nail unit. J. Cutan. Pathol. **29:** 549–556
38 Tar K., Birukova A. A., Csortos C., Bako E., Garcia J. G. and Verin A. D. (2004) Phosphatase 2A is involved in endothelial cell microtubule remodeling and barrier regulation. J. Cell. Biochem. **92:** 534–546
39 Dhamodharan R. and Wadsworth P. (1995) Modulation of microtubule dynamic instability in vivo by brain microtubule associated proteins. J. Cell Sci. **108 (Pt. 4):** 1679–1689
40 Itoh T. J. and Hotani H. (2004) Microtubule dynamics and the regulation by microtubule-associated proteins (MAPs). Biol. Sci. Space **18:** 116–117
41 Bunker J. M., Wilson L., Jordan M. A. and Feinstein S. C. (2004) Modulation of microtubule dynamics by tau in living cells: implications for development and neurodegeneration. Mol. Biol. Cell **15:** 2720–2728
42 Levy S. F., Leboeuf A. C., Massie M. R., Jordan M. A., Wilson L. and Feinstein S. C. (2005) Three- and four-repeat tau regulate the dynamic instability of two distinct microtubule subpopulations in qualitatively different manners. Implications for neurodegeneration. J. Biol. Chem. **280:** 13520–13528
43 Noetzel T. L., Drechsel D. N., Hyman A. A. and Kinoshita K. (2005) A comparison of the ability of XMAP215 and tau to inhibit the microtubule destabilizing activity of XKCM1. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **360:** 591–594
44 Haque N., Gong C. X., Sengupta A., Iqbal K. and Grundke-Iqbal I. (2004) Regulation of microtubule-associated proteins, protein kinases and protein phosphatases during differentiation of SY5Y cells. Brain Res. Mol. Brain Res. **129:** 163–170
45 Fang D., Hallman J., Sangha N., Kute T. E., Hammarback J. A., White W. L. et al. (2001) Expression of microtubule-associated protein 2 in benign and malignant melanocytes: implications for differentiation and progression of cutaneous melanoma. Am. J. Pathol. **158:** 2107–2115
46 Soltani M. H., Pichardo R., Song Z., Sangha N., Camacho F., Satyamoorthy K. et al. (2005) Microtubule-associated protein 2, a marker of neuronal differentiation, induces mitotic defects, inhibits growth of melanoma cells and predicts metastatic potential of cutaneous melanoma. Am. J. Pathol. **166:** 1841–1850
47 Chen J. Y., Chang Y. L., Yu Y. C., Chao C. C., Kao H. W., Wu C. T. et al. (2004) Specific induction of the high-molecular-weight microtubule-associated protein 2 (hmw-MAP2) by betel quid extract in cultured oral keratinocytes: clinical implications in betel quid-associated oral squamous cell carcinoma (OSCC). Carcinogenesis **25:** 269–276
48 Ovechkina Y. and Wordeman L. (2003) Unconventional motoring: an overview of the Kin C and Kin I kinesins. Traffic **4:** 367–375
49 Lawrence C. J., Dawe R. K., Christie K. R., Cleveland D. W., Dawson S. C., Endow S. A. et al. (2004) A standardized kinesin nomenclature. J. Cell. Biol. **167:** 19–22
50 Desai A., Verma S., Mitchison T. J. and Walczak C. E. (1999) Kin I kinesins are microtubule-destabilizing enzymes. Cell **96:** 69–78
51 Walczak C. E., Mitchison T. J. and Desai A. (1996) XKCM1: a Xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. Cell **84:** 37–47
52 Kline-Smith S. L. and Walczak C. E. (2002) The microtubule-destabilizing kinesin XKCM1 regulates microtubule dynamic instability in cells. Mol. Biol. Cell **13:** 2718–2731
53 Holmfeldt P., Stenmark S. and Gullberg M. (2004) Differential functional interplay of TOGp/XMAP215 and the KinI kinesin MCAK during interphase and mitosis. EMBO J. **23:** 627–637
54 Mennella V., Rogers G. C., Rogers S. L., Buster D. W., Vale R. D. and Sharp D. J. (2005) Functionally distinct kinesin-13 family members cooperate to regulate microtubule dynamics during interphase. Nat. Cell Biol. **7:** 235–245
55 Ligon L. A., Shelly S. S., Tokito M. and Holzbaur E. L. (2003) The microtubule plus-end proteins EB1 and dynactin have differential effects on microtubule polymerization. Mol. Biol. Cell **14:** 1405–1417
56 Reilein A. and Nelson W. J. (2005) APC is a component of an organizing template for cortical microtubule networks. Nat. Cell Biol. **7:** 463–473
57 Ohkura H., Garcia M. A. and Toda T. (2001) Dis1/TOG universal microtubule adaptors – one MAP for all? J. Cell Sci. **114** 3805–3812
58 Galjart N. (2005) CLIPs and CLASPs and cellular dynamics. Nat. Rev. Mol. Cell. Biol. **6:** 487–498
59 Kodama A., Karakesisoglou I., Wong E., Vaezi A. and Fuchs E. (2003) ACF7: an essential integrator of microtubule dynamics. Cell **115:** 343–354
60 Sapir T., Elbaum M. and Reiner O. (1997) Reduction of microtubule catastrophe events by LIS1, platelet-activating factor acetylhydrolase subunit. EMBO J. **16:** 6977–69784
61 Rogers S. L., Rogers G. C., Sharp D. J. and Vale R. D. (2002) Drosophila EB1 is important for proper assembly, dynamics and positioning of the mitotic spindle. J. Cell Biol. **158:** 873–884
62 Tirnauer J. S., Grego S., Salmon E. D. and Mitchison T. J. (2002) EB1-microtubule interactions in Xenopus egg extracts: role of EB1 in microtubule stabilization and mechanisms of targeting to microtubules. Mol. Biol. Cell **13:** 3614–3626
63 Wen Y., Eng C. H., Schmoranzer J., Cabrera-Poch N., Morris E. J., Chen M. et al. (2004) EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. Nat. Cell Biol. **6:** 820–830
64 Gard D. L. and Kirschner M. W. (1987) A microtubule-associated protein from Xenopus eggs that specifically promotes assembly at the plus-end. J. Cell Biol. **105:** 2203–2215
65 Charrasse S., Schroeder M., Gauthier-Rouviere C., Ango F., Cassimeris L., Gard D. L. et al. (1998) The TOGp protein is a new human microtubule-associated protein homologous to the Xenopus XMAP215. J. Cell Sci. **111 (Pt. 10):** 1371–1383
66 Tournebize R., Popov A., Kinoshita K., Ashford A. J., Rybina S., Pozniakovsky A. et al. (2000) Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in Xenopus egg extracts. Nat. Cell. Biol. **2:** 13–19
67 Severin F., Habermann B., Huffaker T. and Hyman T. (2001) Stu2 promotes mitotic spindle elongation in anaphase. J. Cell Biol. **153:** 435–442
68 Carvalho P., Gupta M. L. Jr., Hoyt M. A. and Pellman D. (2004) Cell cycle control of kinesin-mediated transport of Bik1 (CLIP-

170) regulates microtubule stability and dynein activation. Dev. Cell **6:** 815–829
69 Komarova Y. A., Akhmanova A. S., Kojima S., Galjart N. and Borisy G. G. (2002) Cytoplasmic linker proteins promote microtubule rescue in vivo. J. Cell. Biol. **159:** 589–599
70 Arnal I., Heichette C., Diamantopoulos G. S. and Chretien D. (2004) CLIP-170/tubulin-curved oligomers coassemble at microtubule ends and promote rescues. Curr. Biol. **14:** 2086–2095
71 Goodson H. V., Skube S. B., Stalder R., Valetti C., Kreis T. E., Morrison E. E. et al. (2003) CLIP-170 interacts with dynactin complex and the APC-binding protein EB1 by different mechanisms. Cell. Motil. Cytoskeleton **55:** 156–173
72 Sheeman B., Carvalho P., Sagot I., Geiser J., Kho D., Hoyt M. A. et al. (2003) Determinants of S. cerevisiae dynein localization and activation: implications for the mechanism of spindle positioning. Curr. Biol. **13:** 364–372
73 Lansbergen G., Komarova Y., Modesti M., Wyman C., Hoogenraad C. C., Goodson H. V. et al. (2004) Conformational changes in CLIP-170 regulate its binding to microtubules and dynactin localization. J. Cell. Biol. **166:** 1003–1014
74 Fukata M., Watanabe T., Noritake J., Nakagawa M., Yamaga M., Kuroda S. et al. (2002) Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell **109:** 873–885
75 Noritake J., Watanabe T., Sato K., Wang S. and Kaibuchi K. (2005) IQGAP1: a key regulator of adhesion and migration. J. Cell Sci. **118:** 2085–2092
76 Mimori-Kiyosue Y., Grigoriev I., Lansbergen G., Sasaki H., Matsui C., Severin F. et al. (2005) CLASP1 and CLASP2 bind to EB1 and regulate microtubule plus-end dynamics at the cell cortex. J. Cell. Biol. **168:** 141–153
77 Wittmann T. and Waterman-Storer C. M. (2005) Spatial regulation of CLASP affinity for microtubules by Rac1 and GSK3{beta} in migrating epithelial cells. J. Cell. Biol. **169:** 929–939
78 Rubin C. I. and Atweh G. F. (2004) The role of stathmin in the regulation of the cell cycle. J. Cell. Biochem. **93:** 242–250
79 Howell B., Larsson N., Gullberg M. and Cassimeris L. (1999) Dissociation of the tubulin-sequestering and microtubule catastrophe-promoting activities of oncoprotein 18/stathmin. Mol. Biol. Cell **10:** 105–118
80 Curmi P. A., Gavet O., Charbaut E., Ozon S., Lachkar-Colmerauer S., Manceau V. et al. (1999) Stathmin and its phosphoprotein family: general properties, biochemical and functional interaction with tubulin. Cell Struct. Funct. **24:** 345–357.
81 Luo X. N., Mookerjee B., Ferrari A., Mistry S. and Atweh G. F. (1994) Regulation of phosphoprotein p18 in leukemic cells. Cell cycle regulated phosphorylation by p34cdc2 kinase. J. Biol. Chem. **269:** 10312–10318
82 Brattsand G., Marklund U., Nylander K., Roos G. and Gullberg M. (1994) Cell-cycle-regulated phosphorylation of oncoprotein 18 on Ser16, Ser25 and Ser38. Eur. J. Biochem. **220:** 359–368
83 Daub H., Gevaert K., Vandekerckhove J., Sobel A. and Hall A. (2001) Rac/Cdc42 and p65PAK regulate the microtubule-destabilizing protein stathmin through phosphorylation at serine 16. J. Biol. Chem. **276:** 1677–1680
84 Bokoch G. M. (2003) Biology of the p21-activated kinases. Annu. Rev. Biochem. **72:** 743–781
85 Ahn J., Murphy M., Kratowicz S., Wang A., Levine A. J. and George D. L. (1999) Down-regulation of the stathmin/Op18 and FKBP25 genes following p53 induction. Oncogene **18:** 5954–5958
86 Polager S. and Ginsberg D. (2003) E2F mediates sustained G2 arrest and down-regulation of Stathmin and AIM-1 expression in response to genotoxic stress. J. Biol. Chem. **278:** 1443–1449
87 Melhem R., Hailat N., Kuick R. and Hanash S. M. (1997) Quantitative analysis of Op18 phosphorylation in childhood acute leukemia. Leukemia **11:** 1690–1695
88 Bieche I., Lachkar S., Becette V., Cifuentes-Diaz C., Sobel A., Lidereau R. et al. (1998) Overexpression of the stathmin gene in a subset of human breast cancer. Br. J. Cancer **78:** 701–709
89 Curmi P. A., Nogues C., Lachkar S., Carelle N., Gonthier M. P., Sobel A. et al. (2000) Overexpression of stathmin in breast carcinomas points out to highly proliferative tumours. Br. J. Cancer **82:** 142–150
90 Howell B., Odde D. J. and Cassimeris L. (1997) Kinase and phosphatase inhibitors cause rapid alterations in microtubule dynamic instability in living cells. Cell. Motil. Cytoskeleton **38:** 201–214
91 Belmont L. D., Hyman A. A., Sawin K. E. and Mitchison T. J. (1990) Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. Cell **62:** 579–589
92 Verde F., Labbe J. C., Doree M. and Karsenti E. (1990) Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of Xenopus eggs. Nature **343:** 233–238
93 Verde F., Dogterom M., Stelzer E., Karsenti E. and Leibler S. (1992) Control of microtubule dynamics and length by cyclin A- and cyclin B-dependent kinases in Xenopus egg extracts. J. Cell. Biol. **118:** 1097–1108
94 Smith D. (2003) Cdk5 in neuroskeletal dynamics. Neurosignals **12:** 239–251
95 Gorbsky G. J. (2004) Mitosis: MCAK under the aura of Aurora B. Curr. Biol. **14:** R346–348
96 Raftopoulou M. and Hall A. (2004) Cell migration: Rho GTPases lead the way. Dev. Biol. **265:** 23–32
97 Yoshida, H., Hastie C. J., McLauchlan H., Cohen P. and Goedert M. (2004) Phosphorylation of microtubule-associated protein tau by isoforms of c-Jun N-terminal kinase (JNK). J. Neurochem. **90:** 352–358
98 Goold R. G. and Gordon-Weeks P. R. (2004) Glycogen synthase kinase 3beta and the regulation of axon growth. Biochem. Soc. Trans. **32:** 809–811
99 Goold R. G. and Gordon-Weeks P. R. (2005) The MAP kinase pathway is upstream of the activation of GSK3beta that enables it to phosphorylate MAP1B and contributes to the stimulation of axon growth. Mol. Cell Neurosci. **28** 524–534
100 Wittmann T., Bokoch G. M. and Waterman-Storer C. M. (2004) Regulation of microtubule destabilizing activity of Op18/stathmin downstream of Rac1. J. Biol. Chem. **279:** 6196–6203
101 Cohen P. T. (1997) Novel protein serine/threonine phosphatases: variety is the spice of life. Trends Biochem. Sci. **22:** 245–251
102 Tournebize R., Andersen S. S., Verde F., Doree M., Karsenti E. and Hyman A. A. (1997) Distinct roles of PP1 and PP2A-like phosphatases in control of microtubule dynamics during mitosis. EMBO J. **16:** 5537–5549
103 Hiraga A. and Tamura S. (2000) Protein phosphatase 2A is associated in an inactive state with microtubules through 2A1-specific interaction with tubulin. Biochem. J. **346 Pt2:** 433–439
104 Sontag E. (2001) Protein phosphatase 2A: the Trojan Horse of cellular signaling. Cell Signal. **13:** 7–16
105 Merrick S. E., Demoise D. C. and Lee V. M. (1996) Site-specific dephosphorylation of tau protein at Ser202/Thr205 in response to microtubule depolymerization in cultured human neurons involves protein phosphatase 2A. J. Biol. Chem. **271:** 5589–5594
106 Sontag E., Nunbhakdi-Craig V., Lee G., Brandt R., Kamibayashi C., Kuret J. et al. (1999) Molecular interactions among protein phosphatase 2A, tau and microtubules. Implications for the regulation of tau phosphorylation and the development of tauopathies. J. Biol. Chem. **274:** 25490–25498
107 Kline-Smith S. L. and Walczak C. E. (2004) Mitotic spindle assembly and chromosome segregation: refocusing on microtubule dynamics. Mol. Cell **15:** 317–327
108 Desai A. and Mitchison T. J. (1997) Microtubule polymerization dynamics. Annu. Rev. Cell. Dev. Biol. **13:** 83–117
109 Saxton W. M., Stemple D. L., Leslie R. J., Salmon E. D., Zavortink M. and McIntosh J. R. (1984) Tubulin dynamics in cultured mammalian cells. J. Cell. Biol. **99:** 2175–2186

110 Zhai Y., Kronebusch P. J., Simon P. M. and Borisy G. G. (1996) Microtubule dynamics at the G2/M transition: abrupt breakdown of cytoplasmic microtubules at nuclear envelope breakdown and implications for spindle morphogenesis. J. Cell. Biol. **135:** 201–214

111 Inoue S. and Salmon E. D. (1995) Force generation by microtubule assembly/disassembly in mitosis and related movements. Mol. Biol. Cell **6:** 1619–1640

112 McIntosh J. R., Grishchuk E. L. and West R. R. (2002) Chromosome-microtubule interactions during mitosis. Annu. Rev. Cell. Dev. Biol. **18:** 193–219

113 Wollman, R., Cytrynbaum E. N., Jones J. T., Meyer T., Scholey J. M. and Mogilner A. (2005) Efficient chromosome capture requires a bias in the 'search-and-capture' process during mitotic-spindle assembly. Curr. Biol. **15:** 828–832

114 McDonald K. L., O'Toole E. T., Mastronarde D. N. and McIntosh J. R. (1992) Kinetochore microtubules in PTK cells. J. Cell. Biol. **118:** 369–383

115 Rusan N. M., Fagerstrom C. J., Yvon A. M. and Wadsworth P. (2001) Cell cycle-dependent changes in microtubule dynamics in living cells expressing green fluorescent protein-alpha tubulin. Mol. Biol. Cell **12:** 971–980

116 Wittmann T., Boleti H., Antony C., Karsenti E. and Vernos I. (1998) Localization of the kinesin-like protein Xklp2 to spindle poles requires a leucine zipper, a microtubule-associated protein and dynein. J. Cell. Biol. **143:** 673–685

117 Gordon M. B., Howard L. and Compton D. A. (2001) Chromosome movement in mitosis requires microtubule anchorage at spindle poles. J. Cell. Biol. **152:** 425–434

118 Levesque A. A., Howard L., Gordon M. B. and Compton D. A. (2003) A functional relationship between NuMA and kid is involved in both spindle organization and chromosome alignment in vertebrate cells. Mol. Biol. Cell **14:** 3541–3552

119 Di Fiore B., Ciciarello M. and Lavia P. (2004) Mitotic functions of the Ran GTPase network: the importance of being in the right place at the right time. Cell Cycle **3:** 305–313

120 McEwen, B. F., Chan G. K., Zubrowski B., Savoian M. S., Sauer M. T. and Yen T. J. (2001) CENP-E is essential for reliable bioriented spindle attachment, but chromosome alignment can be achieved via redundant mechanisms in mammalian cells. Mol. Biol. Cell **12:** 2776–2789

121 Andrews P. D., Ovechkina Y., Morrice N., Wagenbach M., Duncan K., Wordeman L. et al. (2004) Aurora B regulates MCAK at the mitotic centromere. Dev. Cell **6:** 253–268

122 Kline-Smith S. L., Khodjakov A., Hergert P. and Walczak C. E. (2004) Depletion of centromeric MCAK leads to chromosome congression and segregation defects due to improper kinetochore attachments. Mol. Biol. Cell **15:** 1146–1159

123 Lan W., Zhang X., Kline-Smith S. L., Rosasco S. E., Barrett-Wilt G. A., Shabanowitz J. et al. (2004) Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity. Curr. Biol. **14:** 273–286

124 Rieder C. L. and Salmon E. D. (1998) The vertebrate cell kinetochore and its roles during mitosis. Trends Cell Biol. **8:** 310–318

125 Cimini D., Cameron L. A. and Salmon E. D. (2004) Anaphase spindle mechanics prevent mis-segregation of merotelically oriented chromosomes. Curr. Biol. **14:** 2149–2155

126 Higuchi T. and Uhlmann F. (2005) Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation. Nature **433:** 171–176

127 Lauffenburger D. A. and Horwitz A. F. (1996) Cell migration: a physically integrated molecular process. Cell **84:** 359–369

128 Borisy G. G. and Svitkina T. M. (2000) Actin machinery: pushing the envelope. Curr. Opin. Cell Biol. **12:** 104–112

129 Vasiliev J. M., Gelfand I. M., Domnina L. V., Ivanova O. Y., Komm S. G. and Olshevskaja L. V. (1970) Effect of colcemid on the locomotory behaviour of fibroblasts. J. Embryol. Exp. Morphol. **24:** 625–640

130 Kirschner M. and Mitchison T. (1986) Beyond self-assembly: from microtubules to morphogenesis. Cell **45:** 329–342

131 Schuyler S. C. and Pellman D. (2001) Search, capture and signal: games microtubules and centrosomes play. J. Cell Sci. **114:** 247–255

132 Mimori-Kiyosue Y. and Tsukita S. (2003) 'Search-and-capture' of microtubules through plus-end-binding proteins (+TIPs). J. Biochem. (Tokyo) **134:** 321–326

133 Gundersen G. G. and Bulinski J. C. (1986) Microtubule arrays in differentiated cells contain elevated levels of a post-translationally modified form of tubulin. Eur. J. Cell Biol. **42:** 288–294

134 Gundersen G. G. and Bulinski J. C. (1988) Selective stabilization of microtubules oriented toward the direction of cell migration. Proc. Natl. Acad. Sci. USA **85:** 5946–5950

135 Gundersen G. G., Khawaja S. and Bulinski J. C. (1989) Generation of a stable, posttranslationally modified microtubule array is an early event in myogenic differentiation. J. Cell. Biol. **109:** 2275–2288

136 Cook T. A., Nagasaki T. and Gundersen G. G. (1998) Rho guanosine triphosphatase mediates the selective stabilization of microtubules induced by lysophosphatidic acid. J. Cell. Biol. **141:** 175–185

137 Palazzo, A. F., Cook T. A., Alberts A. S. and Gundersen G. G. (2001) mDia mediates Rho-regulated formation and orientation of stable microtubules. Nat. Cell Biol. **3:** 723–729

138 Palazzo A. F., Eng C. H., Schlaepfer D. D., Marcantonio E. E. and Gundersen G. G. (2004) Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. Science **303:** 836–839

139 Infante A. S., Stein M. S., Zhai Y., Borisy G. G. and Gundersen G. G. (2000) Detyrosinated (Glu) microtubules are stabilized by an ATP-sensitive plus-end cap. J. Cell Sci. **113 (Pt. 22):** 3907–3919

140 Westermann S. and Weber K. (2003) Post-translational modifications regulate microtubule function. Nat. Rev. Mol. Cell. Biol. **4:** 938–947

141 Yvon A. M., Walker J. W., Danowski B., Fagerstrom C., Khodjakov A. and Wadsworth P. (2002) Centrosome reorientation in wound-edge cells is cell type specific. Mol. Biol. Cell **13:** 1871–1880

142 Ballestrem C., Magid N., Zonis J., Shtutman M. and Bershadsky A. (2004) Interplay between the actin cytoskeleton, focal adhesions and microtubules. In: Cell Motility: From Molecules to Organisms, pp. 75–99, Ridley A.P., Peckham M. and Clark P. (eds.), John Wiley and Sons

143 Bershadsky A., Chausovsky A., Becker E., Lyubimova A. and Geiger B. (1996) Involvement of microtubules in the control of adhesion-dependent signal transduction. Curr. Biol. **6:** 1279–1289

144 Ren X. D., Kiosses W. B. and Schwartz M. A. (1999) Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO J. **18:** 578–585

145 Kirchner J., Kam Z., Tzur G., Bershadsky A. D. and Geiger B. (2003) Live-cell monitoring of tyrosine phosphorylation in focal adhesions following microtubule disruption. J. Cell Sci. **116:** 975–986

146 Ballestrem C., Wehrle-Haller B., Hinz B. and Imhof B. A. (2000) Actin-dependent lamellipodia formation and microtubule-dependent tail retraction control-directed cell migration. Mol. Biol. Cell **11:** 2999–3012

147 Waterman-Storer C. M., Worthylake R. A., Liu B. P., Burridge K. and Salmon E. D. (1999) Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. Nat. Cell Biol. **1:** 45–50

148 Cau J., Faure S., Comps M., Delsert C. and Morin N. (2001) A novel p21-activated kinase binds the actin and microtubule networks and induces microtubule stabilization. J. Cell Biol. **155:** 1029–1042

149 Wittmann T., Bokoch G. M. and Waterman-Storer C. M. (2003) Regulation of leading edge microtubule and actin dynamics downstream of Rac1. J. Cell Biol. **161:** 845–851
150 Wittmann T. and Waterman-Storer C. M. (2001) Cell motility: can Rho GTPases and microtubules point the way? J. Cell Sci. **114:** 3795–803
151 Kaverina I., Krylyshkina O. and Small J. V. (1999) Microtubule targeting of substrate contacts promotes their relaxation and dissociation. J. Cell Biol. **146:** 1033–1044
152 Krylyshkina O., Anderson K. I., Kaverina I., Upmann I., Manstein D. J., Small J. V. et al. (2003) Nanometer targeting of microtubules to focal adhesions. J. Cell Biol. **161:** 853–859
153 Krylyshkina O., Kaverina I., Kranewitter W., Steffen W., Alonso M. C., Cross R. A. et al. (2002) Modulation of substrate adhesion dynamics via microtubule targeting requires kinesin-1. J. Cell Biol. **156:** 349–359
154 Ezratty E. J., Partridge M. A. and Gundersen G. G. (2005) Microtubule-induced focal adhesion disassembly is mediated by dynamin and focal adhesion kinase. Nat. Cell Biol. **7:** 581–590
155 Mikhailov A. and Gundersen G. G. (1998) Relationship between microtubule dynamics and lamellipodium formation revealed by direct imaging of microtubules in cells treated with nocodazole or taxol. Cell Motil. Cytoskeleton **41:** 325–340
156 Wadsworth P. (1999) Regional regulation of microtubule dynamics in polarized, motile cells. Cell Motil. Cytoskeleton **42:** 48–59
157 Salaycik K. J., Fagerstrom C. J., Murthy K., Tulu U. S. and Wadsworth P. (2005) Quantification of microtubule nucleation, growth and dynamics in wound edge cells. J. Cell Sci. 118: 4113–4122
158 Gotoh Y., Nishida E., Matsuda S., Shiina N., Kosako H., Shiokawa K. et al. (1991) In vitro effects on microtubule dynamics of purified Xenopus M phase-activated MAP kinase. Nature **349:** 251–254
159 Guadagno T. M. and Ferrell J. E. Jr. (1998) Requirement for MAPK activation for normal mitotic progression in Xenopus egg extracts. Science **282:** 1312–1315
160 Roberts E. C., Shapiro P. S., Nahreini T. S., Pages G., Pouyssegur J. and Ahn N. G. (2002) Distinct cell cycle timing requirements for extracellular signal-regulated kinase and phosphoinositide 3-kinase signaling pathways in somatic cell mitosis. Mol. Cell. Biol. **22:** 7226–7241
161 Horne M. M. and Guadagno T. M. (2003) A requirement for MAP kinase in the assembly and maintenance of the mitotic spindle. J. Cell Biol. **161:** 1021–1028
162 Fukata M., Nakagawa M. and Kaibuchi K. (2003) Roles of Rho-family GTPases in cell polarisation and directional migration. Curr. Opin. Cell Biol. **15:** 590–597
163 Yasuda S., Oceguera-Yanez F., Kato T., Okamoto M., Yonemura S., Terada Y. et al. (2004) Cdc42 and mDia3 regulate microtubule attachment to kinetochores. Nature **428:** 767–771
164 Narumiya S., Oceguera-Yanez F. and Yasuda S. (2004) A new look at Rho GTPases in cell cycle: role in kinetochore-microtubule attachment. Cell Cycle **3:** 855–857
165 Li F., Ambrosini G., Chu E. Y., Plescia J., Tognin S., Marchisio P. C. et al. (1998) Control of apoptosis and mitotic spindle checkpoint by survivin. Nature **396:** 580–584
166 Fortugno P., Wall N. R., Giodini A., O'Connor D. S., Plescia J., Padgett K. M. et al. (2002) Survivin exists in immunochemically distinct subcellular pools and is involved in spindle microtubule function. J. Cell Sci. **115:** 575–885
167 Giodini A., Kallio M. J., Wall N. R., Gorbsky G. J., Tognin S., Marchisio P. C. et al. (2002) Regulation of microtubule stability and mitotic progression by survivin. Cancer Res. **62:** 2462–2467
168 Ling X., Bernacki R. J., Brattain M. G. and Li F. (2004) Induction of survivin expression by taxol (paclitaxel) is an early event, which is independent of taxol-mediated G2/M arrest. J. Biol. Chem. **279:** 15196–15203
169 Bourgarel-Rey V., Vallee S., Rimet O., Champion S., Braguer D., Desobry A. et al. (2001) Involvement of nuclear factor kappaB in c-Myc induction by tubulin polymerization inhibitors. Mol. Pharmacol. **59:** 1165–1170
170 Huang Y., Fang Y., Wu J., Dziadyk J. M., Zhu X., Sui M. et al. (2004) Regulation of Vinca alkaloid-induced apoptosis by NF-kappaB/IkappaB pathway in human tumor cells. Mol. Cancer Ther. **3:** 271–277
171 Mistry P., Deacon K., Mistry S., Blank J. and Patel R. (2004) NF-kappaB promotes survival during mitotic cell cycle arrest. J. Biol. Chem. **279:** 1482–1490
172 Reszka A. A., Seger R., Diltz C. D., Krebs E. G. and Fischer E. H. (1995) Association of mitogen-activated protein kinase with the microtubule cytoskeleton. Proc. Natl. Acad. Sci. USA **92:** 8881–8885
173 Nagata K., Puls A., Futter C., Aspenstrom P., Schaefer E., Nakata T. et al. (1998) The MAP kinase kinase kinase MLK2 colocalizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3. EMBO J. **17:** 149–158
174 Bouillet P., Metcalf D., Huang D. C., Tarlinton D. M., Kay T. W., Kontgen F. et al.(1999) Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis and to preclude autoimmunity. Science **286:** 1735–1738
175 Puthalakath H., Huang D. C., O'Reilly L. A., King S. M. and Strasser A. (1999) The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. Mol. Cell **3:** 287–296
176 Marani M., Tenev T., Hancock D., Downward J. and Lemoine N. R. (2002) Identification of novel isoforms of the BH3 domain protein Bim which directly activate Bax to trigger apoptosis. Mol. Cell. Biol. **22:** 3577–3589
177 Giannakakou, P., Sackett D. L., Ward Y., Webster K. R., Blagosklonny M. V. and Fojo T. (2000) p53 is associated with cellular microtubules and is transported to the nucleus by dynein. Nat. Cell Biol. **2:** 709–717
178 Giannakakou P., Robey R., Fojo T. and Blagosklonny M. V. (2001) Low concentrations of paclitaxel induce cell type-dependent p53, p21 and G1/G2 arrest instead of mitotic arrest: molecular determinants of paclitaxel-induced cytotoxicity. Oncogene **20:** 3806–3813
179 Giannakakou P., Nakano M., Nicolaou K. C., O'Brate A., Yu J., Blagosklonny M. V. et al. (2002) Enhanced microtubule-dependent trafficking and p53 nuclear accumulation by suppression of microtubule dynamics. Proc. Natl. Acad. Sci. USA **99:** 10855–10860
180 Galigniana M. D., Harrell J. M., O'Hagen H. M., Ljungman M. and Pratt W. B. (2004) Hsp90-binding immunophilins link p53 to dynein during p53 transport to the nucleus. J. Biol. Chem. **279:** 22483–22489
181 Pourroy B., Carre M., Honore S., Bourgarel-Rey V., Kruczynski A., Briand C. et al. (2004) Low concentrations of vinflunine induce apoptosis in human SK-N-SH neuroblastoma cells through a postmitotic G1 arrest and a mitochondrial pathway. Mol. Pharmacol. **66:** 580–591
182 Guzik B. W. and Goldstein L. S. (2004) Microtubule-dependent transport in neurons: steps towards an understanding of regulation, function and dysfunction. Curr. Opin. Cell Biol. **16:** 443–450
183 Hirokawa, N. and Takemura R. (2004) Kinesin superfamily proteins and their various functions and dynamics. Exp. Cell Res. **301:** 50–59
184 Seitz A., Kojima H., Oiwa K., Mandelkow E. M., Song Y. H. and Mandelkow E. (2002) Single-molecule investigation of the interference between kinesin, tau and MAP2c. EMBO J. **21:** 4896–4905
185 Lopez L. A. and Sheetz M. P. (1993) Steric inhibition of cytoplasmic dynein and kinesin motility by MAP2. Cell. Motil. Cytoskeleton **24:** 1–16
186 Jordan M. A. (2002) Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr. Med. Chem. Anti-Canc. Agents **2:** 1–17

187 Honore S., Kamath K., Braguer D., Wilson L., Briand C. and Jordan M. A. (2003) Suppression of microtubule dynamics by discodermolide by a novel mechanism is associated with mitotic arrest and inhibition of tumor cell proliferation. Mol. Cancer Ther. **2:** 1303–1311
188 Kamath, K. and Jordan M. A. (2003) Suppression of microtubule dynamics by epothilone B is associated with mitotic arrest. Cancer Res. **63:** 6026–6031
189 Ngan V. K., Bellman K., Panda D., Hill B. T., Jordan M. A. and Wilson L. (2000) Novel actions of the antitumor drugs vinflunine and vinorelbine on microtubules. Cancer Res. **60:** 5045–5051
190 Ngan V. K., Bellman K., Hill B. T., Wilson L. and Jordan M. A. (2001) Mechanism of mitotic block and inhibition of cell proliferation by the semisynthetic Vinca alkaloids vinorelbine and its newer derivative vinflunine. Mol. Pharmacol. **60:** 225–232
191 Honore S., Kamath K., Braguer D., Horwitz S. B., Wilson L., Briand C. et al. (2004) Synergistic suppression of microtubule dynamics by discodermolide and paclitaxel in non-small cell lung carcinoma cells. Cancer Res. **64:** 4957–4964
192 Okouneva T., Hill B. T., Wilson L. and Jordan M. A. (2003) The effects of vinflunine, vinorelbine and vinblastine on centromere dynamics. Mol. Cancer. Ther. **2:** 427–436
193 Kelling J., Sullivan K., Wilson L. and Jordan M. A. (2003) Suppression of centromere dynamics by Taxol in living osteosarcoma cells. Cancer Res. **63:** 2794–2801
194 Jordan M. A., Toso R. J., Thrower D. and Wilson L. (1993) Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc. Natl. Acad. Sci. USA **90:** 9552–9556
195 Jordan M. A., Wendell K., Gardiner S., Derry W. B., Copp H. and Wilson L. (1996) Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. Cancer Res. **56:** 816–825
196 Li X. and Nicklas R. B. (1995) Mitotic forces control a cell-cycle checkpoint. Nature **373:** 630–632
197 Zhou J., Panda D., Landen J. W., Wilson L. and Joshi H. C. (2002) Minor alteration of microtubule dynamics causes loss of tension across kinetochore pairs and activates the spindle checkpoint. J. Biol. Chem. **277:** 17200–17208
198 Carre M. and Braguer D. (2005) Microtubule damaging agents and apoptosis. In: Microtubules in Health and Disease, Fojo T. (ed.), Humana Press, in press
199 Varbiro G., Veres B., Gallyas F. Jr. and Sumegi B. (2001) Direct effect of Taxol on free radical formation and mitochondrial permeability transition. Free Radic. Biol. Med. **31:** 548–558
200 Andre N., Carre M., Brasseur G., Pourroy B., Kovacic H., Briand C. et al. (2002) Paclitaxel targets mitochondria upstream of caspase activation in intact human neuroblastoma cells. FEBS Lett. **532:** 256–260
201 Goncalves A., Braguer D., Carles G., Andre N., Prevot C. and Briand C. (2000) Caspase-8 activation independent of CD95/CD95-L interaction during paclitaxel-induced apoptosis in human colon cancer cells (HT29-D4). Biochem. Pharmacol. **60:** 1579–1584
202 Carre M., Andre N., Carles G., Borghi H., Brichese L., Briand C. et al. (2002) Tubulin is an inherent component of mitochondrial membranes that interacts with the voltage-dependent anion channel. J. Biol. Chem. **277:** 33664–33669
203 Dallol A., Agathanggelou A., Fenton S. L., Ahmed-Choudhury J., Hesson L., Vos M. D. et al. (2004) RASSF1A interacts with microtubule-associated proteins and modulates microtubule dynamics. Cancer Res. **64:** 4112–4116
204 Liu L., Vo A. and McKeehan W. L. (2005) Specificity of the methylation-suppressed A isoform of candidate tumor suppressor RASSF1 for microtubule hyperstabilization is determined by cell death inducer C19ORF5. Cancer Res. **65:** 1830–1838
205 Mareel M. M., Storme G. A., De Bruyne G. K. and Van Cauwenberge R. M. (1982) Vinblastine, vincristine and vindesine: anti-invasive effect on MO4 mouse fibrosarcoma cells in vitro. Eur. J. Cancer Clin. Oncol. **18:** 199–210
206 Keller H. U. and Zimmermann A. (1986) Shape changes and chemokinesis of Walker 256 carcinosarcoma cells in response to colchicine, vinblastine, nocodazole and taxol. Invasion Metastasis **6:** 33–43
207 Stearns M. E. and Wang M. (1992) Taxol blocks processes essential for prostate tumor cell (PC-3 ML) invasion and metastases. Cancer Res. **52:** 3776–3781
208 Stracke, M. L., Soroush M., Liotta L. A. and Schiffmann E. (1993) Cytoskeletal agents inhibit motility and adherence of human tumor cells. Kidney Int. **43:** 151–157
209 Liao G., Nagasaki T. and Gundersen G. G. (1995) Low concentrations of nocodazole interfere with fibroblast locomotion without significantly affecting microtubule level: implications for the role of dynamic microtubules in cell locomotion. J. Cell Sci. **108 (Pt. 11):** 3473–3483
210 Nikolai G., Niggemann B., Werner M. and Zanker K. S. (1999) Colcemid but not taxol modulates the migratory behavior of human T lymphocytes within 3-D collagen lattices. Immunobiology **201:** 107–119
211 Hegedus, B., Zach J., Czirok A., Lovey J. and Vicsek T. (2004) Irradiation and Taxol treatment result in non-monotonous, dose-dependent changes in the motility of glioblastoma cells. J. Neurooncol. **67:** 147–157
212 Belotti D., Vergani V., Drudis T., Borsotti P., Pitelli M. R., Viale G. et al. (1996) The microtubule-affecting drug paclitaxel has antiangiogenic activity. Clin. Cancer Res. **2:** 1843–1849
213 Folkman J. (1990) What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst. **82:** 4–6
214 Folkman J. (1992) The role of angiogenesis in tumor growth. Semin. Cancer Biol. **3:** 65–71
215 Parkins C. S., Holder A. L., Hill S. A., Chaplin D. J. and Tozer G. M. (2000) Determinants of anti-vascular action by combretastatin A-4 phosphate: role of nitric oxide. Br. J. Cancer. **83:** 811–816
216 Tozer G. M., Kanthou C., Parkins C. S. and Hill S. A. (2002) The biology of the combretastatins as tumour vascular targeting agents. Int. J. Exp. Pathol. **83:** 21–38
217 Ludford R. J. (1936) The action of toxic substances upon the division of normal and malignat cells in vitro and in vivo. Arch. Exp. Zellforsch **18:** 411–441
218 Ludford R. J. (1945) Cochicine in the experimental chemotherapy of cancer. J. Natl. Cancer Inst. (Bethesda) **6:** 89–101
219 Hill S. A., Lonergan S. J., Denekamp J. and Chaplin D. J. (1993) Vinca alkaloids: anti-vascular effects in a murine tumour. Eur. J. Cancer **29A:** 1320–1324
220 Holwell S. E., Hill B. T. and Bibby M. C. (2001) Anti-vascular effects of vinflunine in the MAC 15A transplantable adenocarcinoma model. Br. J. Cancer **84:** 290–295
221 Prise V. E., Honess D. J., Stratford M. R., Wilson J. and Tozer G. M. (2002) The vascular response of tumor and normal tissues in the rat to the vascular targeting agent, combretastatin A-4-phosphate, at clinically relevant doses. Int. J. Oncol. **21:** 717–726
222 Grosios K., Holwell S. E., McGown A. T., Pettit G. R. and Bibby M. C. (1999) In vivo and in vitro evaluation of combretastatin A-4 and its sodium phosphate prodrug. Br. J. Cancer **81:** 1318–1327
223 Tozer G. M., Prise V. E., Wilson J., Cemazar M., Shan S., Dewhirst M. W. et al. (2001) Mechanisms associated with tumor vascular shut-down induced by combretastatin A-4 phosphate: intravital microscopy and measurement of vascular permeability. Cancer Res. **61:** 6413–6422
224 Klauber N., Parangi S., Flynn E., Hamel E. and D'Amato R. J. (1997) Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. Cancer Res. **57:** 81–86
225 Pasquier E., Carre M., Pourroy B., Camoin L., Rebai O., Briand C. et al. (2004) Antiangiogenic activity of paclitaxel

is associated with its cytostatic effect, mediated by the initiation but not completion of a mitochondrial apoptotic signaling pathway. Mol. Cancer Ther. **3:** 1301–1310

226 Pasquier E., Honore S., Pourroy B., Jordan M. A., Lehmann M., Briand C. et al. (2005) Antiangiogenic concentrations of paclitaxel induce an increase in microtubule dynamics in endothelial cells but not in cancer cells. Cancer Res. **65:** 2433–2440

227 Hotchkiss K. A., Ashton A. W., Mahmood R., Russell R. G., Sparano J. A. and Schwartz E. L. (2002) Inhibition of endothelial cell function in vitro and angiogenesis in vivo by docetaxel (Taxotere): association with impaired repositioning of the microtubule organizing center. Mol. Cancer Ther. **1:** 1191–2000

228 Dhamodharan R., Jordan M. A., Thrower D., Wilson L. and Wadsworth P. (1995) Vinblastine suppresses dynamics of individual microtubules in living interphase cells. Mol. Biol. Cell **6:** 1215–1229

229 Yvon A. M., Wadsworth P. and Jordan M. A. (1999) Taxol suppresses dynamics of individual microtubules in living human tumor cells. Mol. Biol. Cell **10:** 947–959

230 Landen J. W., Lang R., McMahon S. J., Rusan N. M., Yvon A. M., Adams A. W. et al. (2002) Noscapine alters microtubule dynamics in living cells and inhibits the progression of melanoma. Cancer Res. **62:** 4109–4114