



Published in final edited form as:

*Drug Discov Today*. 2014 July ; 19(7): 824–829. doi:10.1016/j.drudis.2013.10.022.

## Interphase microtubules: chief casualties in the war on cancer?

Angela Ogden<sup>1</sup>, Padmashree C.G. Rida<sup>1</sup>, Michelle D. Reid<sup>2</sup>, and Ritu Aneja<sup>1,\*</sup>

<sup>1</sup>Department of Biology, Georgia State University, Atlanta, GA 30303, USA

<sup>2</sup>Department of Pathology, Emory University Hospital, Atlanta, GA 30322, USA

### Abstract

Microtubule-targeting agents (MTAs) profoundly affect interphase cells, for example by disrupting axonal transport, transcription, translation, mitochondrial permeability, immune cell function, directional migration and centrosome clustering. This finding is antithetical to the conventionally held notion that MTAs act on mitosis to trigger arrest-mediated apoptotic cell death. Furthermore, the paucity of mitotic cells in patient tumors and lack of correlation of MTA efficacy with tumor proliferation rate provide strong impetus to re-examine the MTA mechanistic basis of action, with an eye toward interphase activities. Whereas targeted antimetabolites have unequivocally failed their promise across clinical studies, MTAs constitute a mainstay of chemotherapy. This paradox necessitates the conclusion that MTAs exert mitosis-independent effects, spurring a dramatic paradigm shift in our understanding of the mode of action of MTAs.

### Keywords

Microtubule-targeting agents; mitosis; microtubules; interphase; druggable targets; centrosome

### Introduction

Old habits die hard, including those of the intellectual variety. It has been an article of faith within the field of cell biology that microtubule-targeting agents (MTAs) work by poisoning mitosis [1]. As a corollary, it has also seemed axiomatic that the small degree of specificity these chemotherapeutics demonstrate for malignant cells derives from the ostensibly accelerated proliferation rate of tumors compared with most nonmalignant tissues. Observations of the breakneck speed at which immortalized cancer cells divide in culture and in xenografts along with their acute sensitivity to these drugs doubtlessly contributed to this notion. The overt mitotic spindle abnormalities these cells manifest with consequent mitotic arrest and death assuredly reinforced this viewpoint as well [2]. Furthermore, the

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\*Corresponding author: Aneja, R. (raneja@gsu.edu).

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**Teaser:** Clinical evidence rejects the age-old hypothesis that the chemotherapeutic efficacy of microtubule-targeting agents relies on antimetabolic action, and recent findings elaborate on the diverse interphasic mechanisms of these drugs.

characteristic side effects of these drugs arise from the susceptibility of rapidly dividing healthy tissues (e.g. hematopoietic and epithelial tissues) to them. The severe gastrointestinal upset, hair loss and neutropenia that plague chemotherapy patients do confirm that these agents target rapidly dividing cells. Despite the fact that MTAs act on mitotic cells and that patient tumors appear highly proliferative, low mitotic indices are frequently observed in solid patient tumors [3]. Prime examples of this phenomenon are well-differentiated neuroendocrine tumors of the gastrointestinal tract and pancreas (grade 1 and 2 tumors per new 2010 World Health Organization guidelines). These tumors have deceptively bland histomorphology and notoriously low mitotic indices [4]; yet, at the time of diagnosis, many have often invaded local tissues, whereas others have metastasized to distant sites. This inescapable inconsistency has recently been brought to light and termed the ‘proliferation rate paradox’ [2]. This strongly substantiated finding therefore demands that we revisit the heretofore timeworn theory that MTAs act exclusively or even primarily on mitotic cells in the clinic.

### **The payoff for MTAs does not come from mitosis and targeting mitosis does not pay off**

Rather than proliferating more quickly than most normal cells, cancer cells only proliferate in an untimely fashion, slowly and surreptitiously over a period of years. Indeed, the doubling times of many types of solid, primary tumors and metastases are in the order of months or even years, versus only hours or days for continuous cell lines, myeloblasts and epithelial cells [3,5]. A decade can pass between the initiating mutation and the ‘birth’ of the parental cell that founds the malignant tumor, with several more years elapsing before metastasis ensues [6]. Despite the typically low proliferation rate of cancer cells in patient tumors, traditional MTAs, such as taxanes, *Vinca* alkaloids and epothilones, often expeditiously shrink the lesion, suggesting that these agents act in a mitosis-independent manner [1]. In support of this notion, proliferation rate was determined to be unassociated with response to docetaxel in breast cancer [7], paclitaxel and vinorelbine in ovarian cancer [8], paclitaxel in non-small-cell lung cancer [9] and epothilone B in glioblastoma [10]. Small-cell carcinoma of the lung, which unlike many other types of cancers has a high mitotic index, should in theory be responsive to MTAs with correspondingly better patient outcomes; however, this has not been demonstrated in clinical trials [11]. Collectively, these findings disaffirm mitosis as a clinically significant target of MTAs in many types of cancer.

Consequently, it is unsurprising that the new fleet of mitosis-targeted chemotherapeutics has generated a wave of disappointment in recent clinical trials [3]. A central motivation for the development of these agents was to attenuate or eliminate the devastating side effects of traditional MTAs. Unfortunately, targeted antimetabolites have thus far not lived up to their promise despite billions of dollars invested in their research [3]. For instance, inhibitors of Aurora kinases, Polo-like kinases and kinesin spindle protein, the major novel antimetabolites, have suffered from low success rates in clinical trials with less than 2% overall response rate, which, dismayingly, was similar to placebo [1]. Although low absorption or bioavailability could underlie the observed ineffectiveness of targeted antimetabolites, several studies have shown that their pharmacokinetic profiles are favorable, and the small fraction

of mitotic cells present in tumors from patients treated with these drugs display their signature effects (e.g. mitotic arrest, chromosome misalignment, monopolar and/or multipolar spindles) [12–19]. Therefore, it appears that most of these drugs in fact successfully reach and act on diverse tumor types, although without considerable oncolysis and with the best outcome often simply ‘stable disease’ [3].

### Interphase cells: the unanticipated victims of MTA attack

Although MTAs are fairly<sup>[s1]</sup> potent in their ability to destroy mitotic cells, there are demonstrably few mitotic cells in most clinically perceptible solid tumors and targeting mitosis is not an effective chemotherapeutic strategy. The inevitable conclusion is that MTAs predominantly target interphase cells in cancer patients. A relatively small but expanding body of research implicates derangement of interphase activities as mechanistically involved in MTA-mediated cytotoxicity. Some unresolved issues and inconsistencies in the literature urge a more thorough investigation of this idea, which is the foremost motivation of this review. Perhaps the most conspicuous evidence for interphase actions of MTAs is the severe impairment of nondividing cells such as neurons, which supports an interphase mechanism of action in this cell type [3]. This neuronal sensitivity accounts for the dose-limiting neuropathies so common after treatment with these chemotherapeutics: approximately one-third of MTA-treated patients experience severe peripheral neuropathy [20]. However, we do not believe that the post-mitotic nature of neurons *ipso facto* proves that MTAs induce neurotoxicity by directly targeting interphase cells – specifically neurons – because the potentially mitotic glial cells surrounding and sustaining neurons are also known to be affected by these drugs [21,22]. The contribution of glia–neuron interactions in neuronal susceptibility to MTAs, nevertheless, represents essentially uncharted waters. Even if these interactions are considerable, the specific interphase actions of MTAs on glial cells have been reported, such as inhibition of kinesin-1-mediated transport of Smad2 to the nucleus in paclitaxel-treated astrocytes [23].

Regardless, there remain ample data along with strong theoretical bases in favor of direct actions of MTAs on neurons, such as characteristically disrupted microtubule dynamic instability after application of MTAs at clinically relevant concentrations and hyper- or depolymerization of microtubules with higher doses [24–27]. In addition, studies of isolated axoplasm reveal that MTAs disrupt anterograde, kinesin-dependent, fast axonal transport, perhaps excluding glia as the complicating factors in this case. Peripheral sensory nerves are especially sensitive to MTAs and they project particularly long, microtubule-laden axons, insinuating that MTAs disrupt axoplasmic transport crucial to axonal survival [29]<sup>[s2]</sup>. Although these nerves can be myelinated, raising the possibility that Schwann cells are targeted, peripheral neuropathy often presents as axonopathy with or without myelinopathy [20], suggesting that axonal dysfunction can occur without overt disease of Schwann cells. Given the debilitating neuropathies suffered by MTA-treated cancer patients, it is unexpected that these drugs have recently found potential application in treating axonopathy and neurotoxicity, such as in models of Alzheimer’s disease and central nervous system (CNS) injury [30,31]. One plausible explanation for this discrepancy is that healthy neurons respond differently to MTAs compared with injured ones, a hypothesis that merits further testing. Altogether, these drugs exhibit unmistakable interphase effects on the neuronal

cytoskeleton, notably in axons, which alters microtubule-mediated transport in a context-dependent manner.

### **Spotlighting the actions of MTAs on cancer cells in interphase: jamming traffic along several routes**

Several groups including ours have consistently demonstrated that MTA treatment perturbs microtubule dynamicity in interphase cells [32]. In addition, MTAs significantly disturb interphase intracellular trafficking of protein and nucleic acid cargo in various types of non-neuronal cancer cells. For instance, taxanes antagonize androgen receptor signaling in prostate cancer cells by thwarting its dynein-mediated trafficking to the nucleus along interphase microtubules [33,34]. Similarly, paclitaxel decreases the velocity of endocytic trafficking of epidermal growth factor receptor and shuttles it to lysosomes situated in the periphery rather than in the perinuclear region in lung carcinoma cells [35]. Paclitaxel and vincristine suppress nuclear accumulation of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) following hypoxia in prostate cancer cells via a mechanism dependent on inhibition of interphase microtubules [36]. By contrast, other studies have shown that dynamicity-suppressing doses of MTAs result in enhanced trafficking of certain cargo along microtubules. For instance, paclitaxel, vincristine and nocodazole promote nuclear accumulation of p53 in lung carcinoma cells [37], as does benomyl in breast cancer cells [38]. Thus, it currently remains unclear how attenuation of microtubule dynamicity selectively promotes or suppresses trafficking of certain factors but not others, which warrants further in-depth exploration. Identification of determinants that confer such specificity could lay the groundwork for uncovering novel interphase targets and might aid rational design of chemotherapeutics that alter the transport of specific (or a specific class of) factors along interphase microtubules.

In addition to alterations in intracellular trafficking, MTAs modulate transcription and translation in cancer cells. In a series of elegant experiments, it was revealed that disruption of interphase microtubules in various types of cancer cells by paclitaxel and vinblastine lowers levels of HIF-1 protein by impairing trafficking of HIF-1 $\alpha$  mRNA along microtubules and inducing its release from polysomes, with subsequent consignment of the mRNA to degradative P-bodies [39]. The involvement of interphase microtubules in transcription factor trafficking and the effects of MTAs on this process are well documented [40]; however, implication of interphase microtubules in the regulation of translation is entirely novel and the ramifications are clearly far-reaching. The mechanisms by which MTAs induce mRNA detachment from ribosomes are completely unknown, and clarification of these pathways would contribute to engineering of next-generation chemotherapeutics.

### **Surveying the MTA trail of destruction: organelle and whole-cell effects**

Altered trafficking of biomolecules by MTAs not only impacts the transcriptional and translational programs of cancer cells but also a host of other activities, including those at the organelle and whole-cell levels. Vesicular traffic from the Golgi apparatus along its microtubule array to the leading edge of the cell is necessary for leading-edge protrusion

[41]. Moreover, Golgi integrity is maintained by the microtubule cytoskeleton [42,43], so it is reasonable to think that MTAs suppress directional migration of cancer cells [41,44]. Furthermore, regulation of actin polymerization and focal adhesion turnover by dynamic interphase microtubules also navigates cell migration [41], which perhaps aids explanation of the antimetastatic effects of MTAs in some cancers. MTAs additionally alter mitochondrial function and ion homeostasis in cancer cells and also modulate immune responses to tumors, arguing for the existence of multiple mitosis-independent mechanisms for these drugs. Regarding calcium homeostasis, MTAs can alter calcium-dependent signaling through mitochondria-dependent mechanisms, the effects of which can be extensive owing to the ubiquity of this divalent cation in signal transduction pathways. For instance, paclitaxel-mediated opening of the mitochondrial permeability transition pore releases calcium into the cytosol and extinguishes its oscillations [45], whereas *Vinca* alkaloids decrease calcium uptake and release by mitochondria [46]. MTAs also demonstrate significant mitotoxicity, perhaps because of the presence of  $\beta$ -tubulin in the voltage-dependent anion channel (VDAC) of the outer mitochondrial membrane [47]. MTAs induce opening of the VDAC-containing mitochondrial permeability transition pore in cancer cells and release of cytochrome c from the mitochondrion with subsequent activation of the caspase cascade [48].

Another particularly attractive notion is that the chief microtubule-organizing centers of cancer cells – centrosomes – are fundamentally different from those in healthy cells. Cancer cells are well-known to often harbor an excessive number of centrosomes, which can also be structurally and functionally abnormal [49–51]. Clustering of centrosomes in interphase cells can be crucial to directional cancer cell migration, as has been hypothesized [51], so MTAs that are also putative declustering drugs (e.g. griseofulvin, noscapinoids, PJ-34) can prove antimetastatic [52,53]. An intriguing hypothesis is that declustering of interphase centrosomes could antagonize the diverse other integral cellular processes discussed in this article. Undoubtedly, these innovative studies illuminate the vast array of means by which MTAs can cripple cells, literally in terms of motility and figuratively in terms of intracellular signaling and metabolism.

### **MTAs tamper with interphase activities to bolster immune attack on tumors**

Finally, MTAs exert substantial immunomodulatory effects that contribute to tumor rejection. For example, in a murine melanoma model, paclitaxel at ultra-low concentrations (i.e. those that do not suppress hematopoiesis) disrupts myeloid-derived suppressor cell recruitment to tumors, impairs the activity of these generally tumor-promoting leukocytes and improves CD8+ T cell effector functions and survival [54]. In fact, improved survival following paclitaxel treatment was completely eliminated by depletion of CD8+ T cells. Taxanes induce cytokine secretion by macrophages as well as cancer cells owing to changes in transcriptional programs. Activated macrophages can then combat tumors themselves or via recruitment and stimulation of dendritic cells, natural killer cells and/or CD8+ T cells that can contribute to this task [55,56]. Treatment of lung cancer patients with paclitaxel selectively depletes CD4+CD25+Foxp3+ regulatory T cell populations and attenuates their activity in an intriguingly tubulin-independent, Bcl2-dependent manner [57]. Inhibition of these anti-inflammatory leukocytes can mitigate the cancer-promoting immunosuppression

that often exists within the tumor microenvironment. Low-dose taxanes, *Vincas* and Etoposide upregulate MHC class I expression and proinflammatory cytokine secretion by ovarian cancer cells, rendering them better targets for immune cells and also augmenting potentially tumoricidal inflammatory mechanisms [56]. Paclitaxel and vinblastine have been shown to improve antigen presentation by dendritic cells [58], although another study reported enhanced presentation only with paclitaxel and not vinblastine [59]. Ultimately, it is unambiguous that MTAs extensively modulate the immune system capacity to respond to tumors in ways that depend on expressly interphase actions (e.g. leukocyte trafficking, transcription, antigen presentation).

## Targeting interphase: the new game plan in the war on cancer

Which of these multitudinous MTA-dependent mechanisms of interference with interphase activities are most crucial in tipping the balance toward tumor rejection is important to identify so that therapies with enhanced specificity and efficacy can be developed. However, it is entirely conceivable that these chemotherapeutics work chiefly in interphase cancer cells by exacting ‘death by a thousand cuts’, because there are undeniably a great number of mechanisms by which MTAs operate, as illustrated in Figure 1. If this is true, then novel targeted therapies could continue to be eclipsed by traditional MTAs. It will also be important to decipher the ways in which interphase-specific cellular processes and structural features in cancer cells (such as those enumerated in Table 1) might be more susceptible to disruption than in healthy cells. Such efforts at identifying druggable differences between normal and cancer cells in interphase are needed to develop the next generation of ‘discriminating’ chemotherapeutics to target tumors more effectively and precisely and thereby reduce the grievous side-effect profiles that remain characteristic of modern chemotherapeutics. There is evidence that cancer cells could be inherently more susceptible to a multitude of perturbations, essentially living on the brink of life or death [60,61]. This ‘extreme lifestyle’ can render cancer cells more vulnerable to chemotherapy in general but it can also see the evolution of treatment-resistant strains. Ultimately, pinpointing the crucial interphase mechanisms of MTAs can guide rational design of a superior class of minimally toxic chemotherapeutics.

## Acknowledgments

We gratefully acknowledge the assistance and support of Trenton X. Millner for the scientific illustrations.

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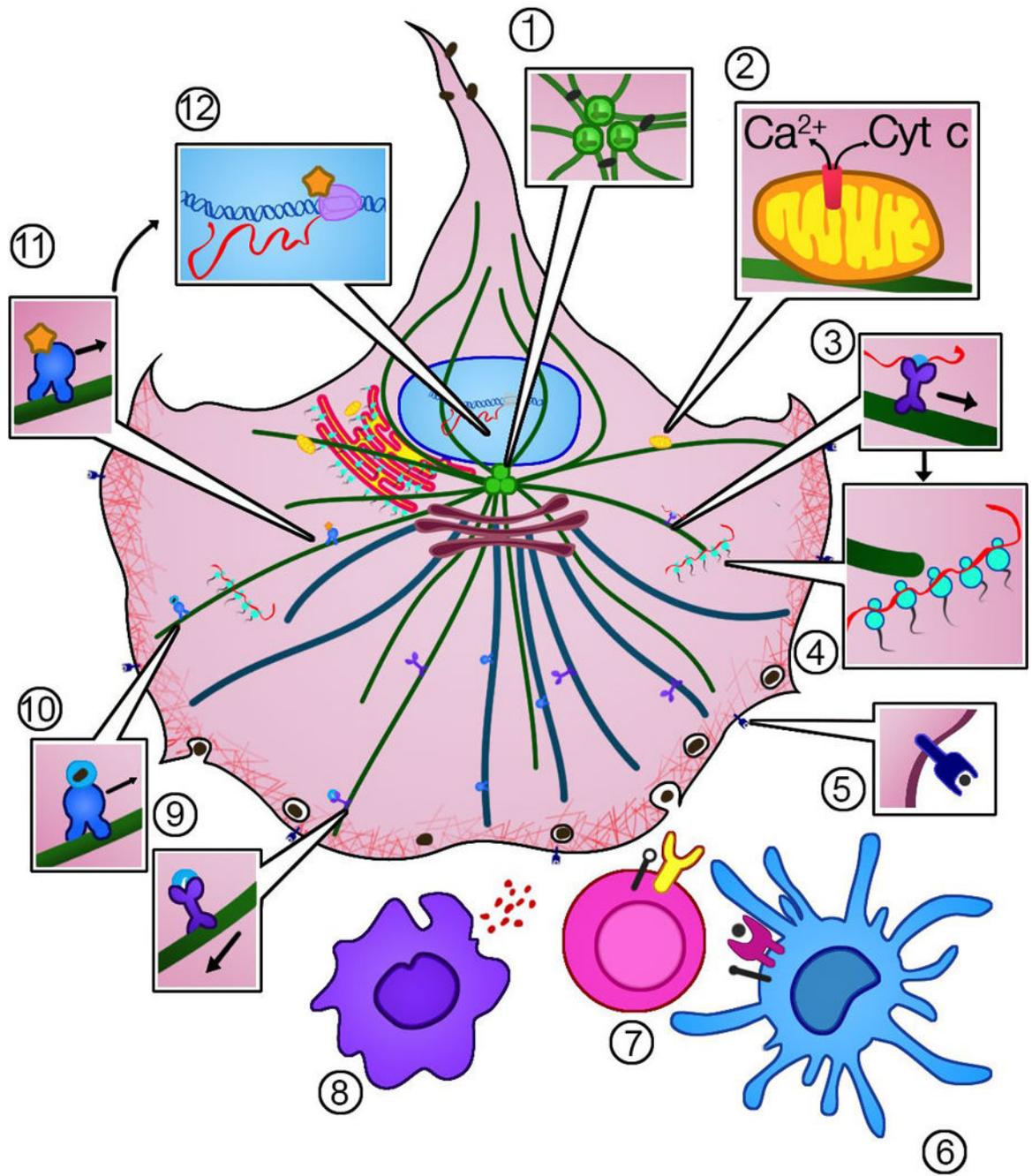
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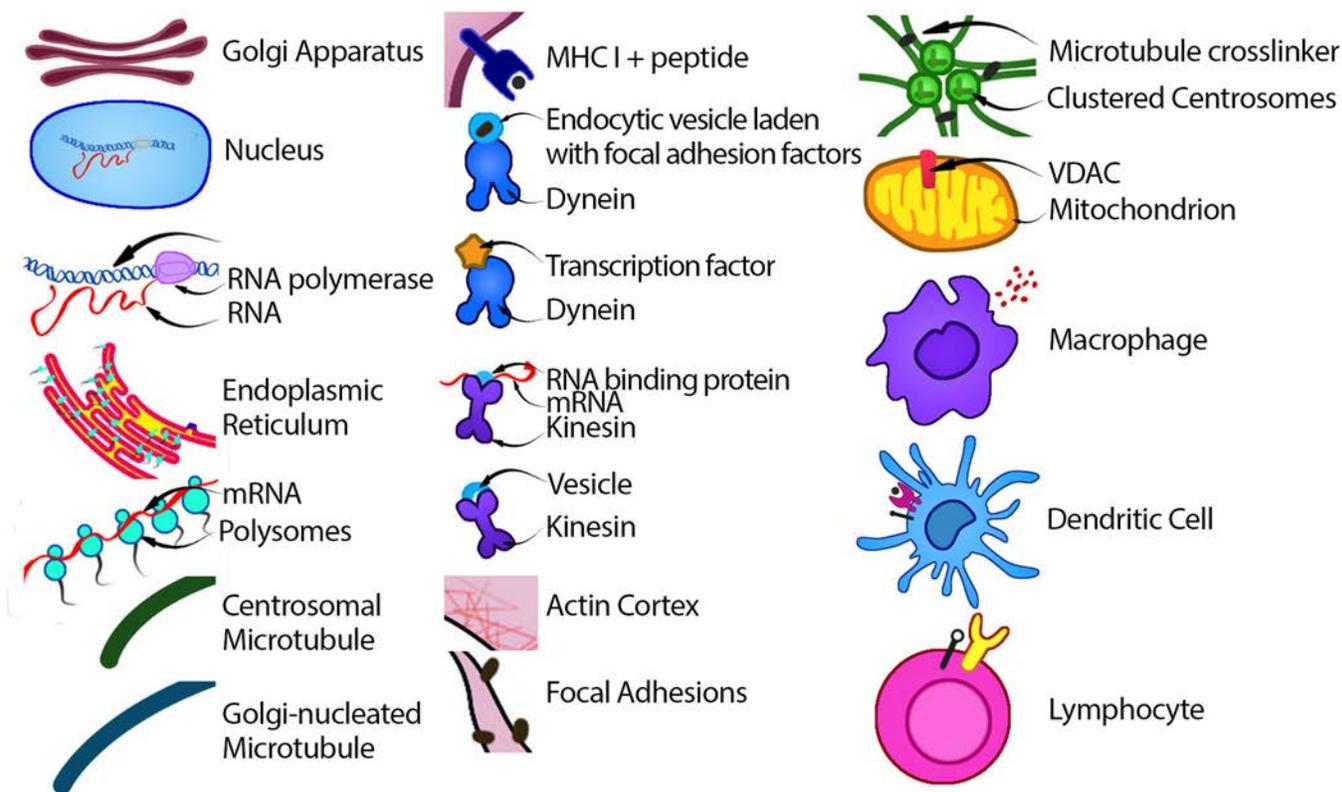
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### Highlights

- Microtubule-targeting agents (MTAs) were once thought to fight cancer via inhibition of mitosis
- Tumor growth is typically slow and novel mitosis-specific inhibitors have failed clinically
- A wealth of evidence suggests that MTAs act primarily on interphase cells in cancer patients
- Newly identified MTA targets include interphase-specific processes, organelles and cells
- Knowledge of these targets and cell culture limitations can guide novel anticancer drug design





**Figure 1. Diverse anticancer interphase activities of microtubule-targeting agents (MTAs)**

(a) <sup>[s4]</sup>Centrosome clustering is antagonized by novel microtubule-binding agents like griseofulvin and noscapinoids, which could impact diverse cellular activities such as Golgi compaction and polarization along with cell polarization and directional migration. (b) MTAs are mitotoxic and induce voltage-dependent anion channel opening with release of  $\text{Ca}^{2+}$  and cytochrome c. (c) MTAs also disrupt delivery of mRNA along interphase microtubule tracks to polysomes and (d) induce mRNA release from polysomes. (e) MTAs improve MHC class I expression by cancer cells, which could render them more 'perceptible' to the immune system, along with increasing activation of (f) dendritic cells, (g) cytotoxic T lymphocytes and (h) macrophages, among other generally proinflammatory, antitumor leukocytes. (i) By impeding vesicular traffic to the cell front, MTAs can inhibit the delivery of vesicles containing actin- and focal-adhesion-regulating factors, and matrix-digesting proteases, which normally promote leading edge protrusion and stromal invasion, respectively. (j) Similarly, interphase microtubule tracks are required for timely endocytosis of focal adhesion components from the leading edge and recycling to the trailing edge to propel directional migration. (k) Finally, MTAs interfere with transcription factor transport by motors to the nucleus with (l) up- or down-regulation of tumor suppressor or oncogenes, respectively.

Abbreviations: MHC, major histocompatibility complex; VDAC, voltage-dependent anion channel.

**Table 1**

Possible differences in interphase processes and subcellular structures between normal and malignant cells, which might be targeted by MTAs or novel chemotherapeutics

Process-level differences	Structural, cytoskeletal and/or organellar differences
Gene expression (transcription and translation)	Microtubules (e.g. tubulin isoforms, degree of dynamicity, threshold for perturbing dynamicity, MAP binding, post-translational modifications, density, organization)
DNA replication	
DNA damage responses	
G1/S and G2/M checkpoints	
Growth-factor-dependent growth	Microfilaments (e.g. treadmilling, actin-binding and bundling proteins, stress fiber subtypes, cellular F-actin content, upstream signaling, centripetal flow, actinomyosin-mediated contractility)
Centrosome or other organelle duplication	
Vesicular trafficking	
Cell adhesion	
Signaling pathways (MAPK/ERK, SAPK/JNK, PI3K/Akt, AMPK, JAK/STAT, NF- $\kappa$ B, Wnt, etc.)	Intermediate filaments (phosphorylation and reorganization; levels of vimentin, keratins, etc.)
Differentiation programs	Centrosomes and centrioles (size, number, structure, microtubule-nucleation capacity, subcellular localization, primary cilium formation, signaling)
Apoptosis, necrosis or other manner of death	
Circadian rhythms	
Metabolic pathways (e.g. oxidative phosphorylation, glycolysis, fermentation, pentose-phosphate pathway)	Golgi (integrity, glycosylation and microtubule-nucleation capacity)
Stress responses	Mitochondria (ROS levels, mtDNA mutations)
Antigen presentation and processing	Nuclear matrix or lamina
Cell-cell interactions	Lysosomes (enzyme and ROS content, pH)

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ERK, extracellular signal-regulated kinase; JAK, Janus kinase; JNK, c-Jun amino-terminal kinase; MAP, microtubule-associated protein; MAPK, mitogen-activated protein kinase; MTA, microtubule-targeting agents; mtDNA, mitochondrial DNA; NF- $\kappa$ B, nuclear factor kappa beta; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; STAT, signal transducer and activator of transcription.