Microtubule-Associated Proteins as Targets in Cancer Chemotherapy

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Abstract
Natural and synthetic compounds that disrupt microtubule dynamics are among the most successful and widely used cancer chemotherapeutic agents. However, lack of reliable markers that predict sensitivity of cancers to these agents and development of resistance remain vexing issues. There is accumulating evidence that a family of cellular proteins that are associated with and alter the dynamics of microtubules can determine sensitivity of cancer cells to microtubule-targeting agents and play a role in tumor cell resistance to these agents. This growing family of microtubule-associated proteins (MAP) includes products of oncopgenes, tumor suppressors, and apoptosis regulators, suggesting that alteration of microtubule dynamics may be one of the critical events in tumorigenesis and tumor progression. The objective of this review is to integrate the knowledge on these seemingly unrelated proteins that share a common function and examine their relevance to microtubule-targeting therapies and highlight MAPs-tubulin-drug interactions as a novel avenue for new drug discovery. Based on the available evidence, we propose that rational microtubule-targeting cancer therapeutic approaches should ideally include proteomic profiling of tumor MAPs before administration of microtubule-stabilizing/destabilizing agents preferentially in combination with agents that modulate the expression of relevant MAPs.

Dynamic instability is an essential and indispensable property of microtubules. This property is evident most notably during assembly of bipolar spindle and segregation of duplicated chromosomes in mitosis. Several natural and synthetic compounds that disrupt microtubule dynamics, and hence block mitosis, are currently in use as cancer chemotherapeutic agents. Variable sensitivity of different cancers and frequent acquisition of resistance by others to these agents has prompted a search for cellular factors and mechanisms that determine the effectiveness of microtubule-targeting agents (1). A better understanding of such factors, mechanisms, and their role in conferring resistance to microtubule-targeting agents is essential for rational use of highly effective, often toxic, microtubule-targeting agents for cancer chemotherapy. In this review, we synthesize the available knowledge on a seemingly disparate group of proteins that, by virtue of binding to microtubules, not only influence the sensitivity of cancer cells to microtubule-targeting agents but also seem to be associated with tumor progression. Proteomic profiles of tumor microtubule-associated proteins (MAP) and an understanding the molecular mechanisms that regulate their expression will help design more effective strategies for the use of microtubule-targeting agents in cancer chemotherapy.

Microtubules

Microtubules are essential components of the cytoskeleton and play a critical role in many cellular processes, including cell division, cell motility, intracellular trafficking, and cell shape maintenance. Composed of αβ-tubulin heterodimers, microtubules are intrinsically dynamic polymers, and their dynamic property is crucial for the assembly of the mitotic spindle and the attachment and movement of chromosomes along the spindle (2, 3). Suppression of microtubule dynamics by microtubule-targeting drugs, such as the Vinca alkaloids and taxanes, can engage the mitotic spindle checkpoint, arresting cell cycle progression at mitosis and eventually leading to apoptosis (4). These drugs, which are in wide use as cancer chemotherapeutic agents, generally bind to one of the two classes of sites on tubulin, the Vinca domain and paclitaxel site. Therefore, concerted efforts are ongoing to identify, design, and develop agents that bind to these and other sites on tubulin and alter microtubule dynamics with minimal toxicity to normal tissues. In addition to their direct involvement in the physical process of mitosis, microtubules also serve as scaffolds for signaling molecules. Thus, sustained modification of signaling routes and changes in the scaffolding properties of microtubules seem to constitute two major processes in the apoptotic response induced by microtubule-interfering agents (reviewed in ref. 5).

Cellular Regulation of Microtubule Dynamics

Intracellular dynamic behavior of microtubules is regulated by a balance between activities of microtubule-stabilizing and microtubule-destabilizing proteins (6) that include, in various cell types, a family of MAPs, tau, oncoprotein stathmin/oncoprotein 18, tumor suppressors BRCA1 and pVHL (von...
Hippel-Lindau syndrome) protein and inhibitor apoptosis protein, survivin, and others (Fig. 1). These seemingly unrelated proteins share a common feature [i.e., they contain tubulin binding domains(s)]. Whereas changes in phosphorylation of some of these proteins are responsible for cell cycle–specific alterations of the microtubule network, changes in levels of expression others seem to correlate with aggressiveness of a variety of human cancers and/or their sensitivity to microtubule-targeting chemotherapeutic agents. This property of MAP binding to tubulin and altering microtubule dynamics in the context of tumor sensitivity to microtubule-targeting agents provides an opportunity to manipulate MAP–tubulin interactions to affect tubulin–drug interactions and clinical outcome in cancer chemotherapy.

**MAPs**

MAPs are a family of proteins that bind to and stabilize microtubules. Whereas MAP4 is expressed ubiquitously, isoforms of MAP1 and MAP2 are expressed primarily in neurons, and MAP7 is restricted to epithelial cells (7). Aberrant expression of MAPs and their relevance to the resistant phenotype of a wide range of malignancies to microtubule-targeting agents have been documented.

**Tau.** Tau is one of the most extensively investigated MAP. It is found primarily in neurons, where its phosphorylated isoforms bind to tubulin to promote polymerization and stabilization of axonal microtubules. Abnormal phosphorylation of tau is associated with Alzheimer’s disease and other neurodegenerative disorders known as tauopathies (8).

Expression of tau in nonneuronal tissues, including breast cancer cells, has been reported (9). Rouzier et al. (9) identified tau as the most differentially expressed gene that is inversely associated with pathologic complete response of breast cancers to preoperative paclitaxel chemotherapy. Consistent with its function in stabilizing microtubules, loss of tau expression sensitizes breast cancer cells to the action of paclitaxel. This suggests that breast cancer patients may be selected for paclitaxel therapy based on low tau expression, and that inhibition of tau could be a strategy to make tumors sensitive to paclitaxel. Although tau is a promising marker of sensitivity to paclitaxel, other mechanisms and pathways of acquiring resistance to microtubule-targeting agents need to be considered.

**MAP2.** MAP2 is found primarily in the dendritic extensions of post-mitotic, terminally differentiated neurons. MAP2 plays a critical role in neurite outgrowth and dendrite development (reviewed in ref. 10). Among neuronal MAPs, MAP2 expression is considered a hallmark of neuronal differentiation (11). Consistent with its microtubule-stabilizing function, expression of MAP2 in nonneuronal cells results in rapid formation of stable microtubule bundles and dendrite-like processes (12).

Variable MAP2 immunoreactivity is found in most pulmonary neuroendocrine carcinomas and in some non–small-cell carcinomas (13), whereas Merkel cell carcinomas show diffuse to focal MAP2 expression. MAP2 is thought to be a valuable ancillary marker in skin tumors suspicious of neuroendocrine origin (14). An inducible, high molecular weight isoform of MAP2 has been proposed as a diagnostic marker in oral squamous cell carcinoma (15).

Fang et al. (16) described induction of juvenile and adult isoforms of MAP2 in cultured metastatic melanoma cells and their expression in benign and primary malignant melanomas. Focal expression of MAP2 was found only in a few metastatic melanomas. Kaplan-Meier survival and multivariate Cox regression analysis showed that patients diagnosed with MAP2+ primary melanomas have significantly better metastatic disease-free survival than those with MAP2− disease. Investigation of the mechanisms that underlie the effect of MAP2 on melanoma progression showed that MAP2 expression in metastatic melanoma cells leads to microtubule stabilization, cell cycle arrest in G2–M phase, and growth inhibition in vitro and in vivo. These data suggest that activation of microtubule-stabilizing proteins in primary cancer cells may inhibit their proliferation and correlate with a delay or inhibition of metastasis (17).

Moreover, the ability to induce/up-regulate MAP2 expression in metastatic melanoma cells with pharmacologic agents makes it an attractive target for therapeutic strategies. Recent studies in our laboratory have shown that in melanoma cells Notch signaling pathways are involved in regulation of MAP2 gene expression (18). Taken together with the observation that small molecule inhibitors of Notch are highly effective in inducing apoptosis of melanoma cells (19), it is tempting to propose that therapeutic approaches that also target pathways involved in activation of MAP2 expression may be highly effective for melanoma.

Expression of MAP2 in other cancers has been associated with sensitivity to microtubule-targeting drugs. Increased expression of MAP2-related peptides has been found in docetaxel-sensitive pancreatic ductal adenocarcinoma, compared with the paclitaxel-refractory pancreatic cancers (20).
Interestingly, proteolysis of MAP2 increases the tendency of tubulin to assemble into microtubules and aggregates in the presence of docetaxel, suggesting that proteolysis of MAP2 might increase microtubule alterations and potentiate the antitumor effect of docetaxel.

MAP4. Alterations in expression of isoforms of the ubiquitously expressed MAP4 have been reported to modulate cancer cell sensitivity to microtubule-interacting drugs. MAP4 phosphorylation and dissociation from microtubules correlate with a decrease in Taxol sensitivity in Taxol-resistant ovarian cancer cell lines (21). In contrast, expression of nonphosphorylated forms of MAP4 is increased in vinblastine-resistant cells (22). Moreover, inhibition of spontaneous growth of p16-null human ovarian cancer cells by expression of p16 is found to be associated with increased MAP4 expression (23). Increased levels of MAP4 and class III β-tubulin, on the other hand, seems to correlate with resistance of human leukemia cells to a microtubule-targeting Epothilone analogue desoxyepothilone B (24). The effects of this ubiquitously expressed MAP seem to be cell type dependent and warrant additional in vivo animal studies in conjunction with microtubule-targeting agents.

Oncogenes and Tumor Suppressors

Stathmin/Oncoprotein 18. Stathmin, originally identified as an oncoprotein, is the founding member of a family of microtubule destabilizing proteins that play a critical role in the regulation of mitosis (25). Activity of stathmin is regulated by phosphorylation at multiple sites (26). Evidence for the role of stathmin in regulation of mitosis came from genetic studies that showed that manipulation of stathmin expression interferes with the progression of cells through mitosis (27). Stathmin is thought to act by two mechanisms: as a tubulin-sequestering protein and as a catastrophe promoter (6, 28).

Increased expression of stathmin has been shown to precede tumor development in the 7,12-dimethylbenz(a)anthracene-induced rat mammary gland carcinogenesis model, raising the possibility that disruption of microtubule dynamics and abnormal mitosis may be critical events in breast cancer development (29). High levels of stathmin expression were also reported in a variety of human malignancies (reviewed in ref. 30). Interestingly, in many of these cancers, a high level of stathmin expression is associated with poor prognosis (31, 32). In human breast cancers, stathmin/oncoprotein 18 levels are positively correlated with a high fraction of aneuploid cells, proliferative cells, tumor size, and histopathologic grade (32).

A role for stathmin in tumorigenesis and malignant phenotype is also supported by the observation that antisense inhibition of stathmin in K562 leukemic cells resulted in abrogation of the malignant phenotype (33). Adenovirus-mediated transfer of anti-stathmin ribozymes in LNCaP prostate cancer cells resulted in a dramatic dose-dependent growth inhibition, accumulation of cells in G2-M phase, and apoptosis (34). In certain cell types, mutations in stathmin that affect its phosphorylation can also contribute to tumorigenesis (35). Thus, stathmin is an attractive target for cancer therapeutics that aim to disrupt microtubule dynamics (30).

In addition, there is a relationship between stathmin expression and sensitivity of tumors to microtubule-targeting agents. Overexpression of stathmin decreases polymerization of microtubules and consequently decreases sensitivity to paclitaxel and, to a lesser extent, to vinblastine. In contrast, stathmin content has no significant effect on the sensitivity to chemotherapeutic drugs that do not target microtubules. Stathmin can affect the action of microtubule-targeting agents by both altering drug binding to microtubules and growth arrest at G2-M phase of the cell cycle (36).

Inhibition of stathmin expression sensitizes K562 leukemic cells to killing by Taxol and seems to protect cells from the effects of the microtubule destabilizing agent vinblastine instead of sensitizing them. Similarly, neuroblastoma cell lines, which are resistant to vincristine, exhibit high levels of stathmin. In contrast, levels of MAP2 were relatively unaltered in these cells (37). Resistance of ovarian carcinoma cells to microtubule-targeting drugs has also been reported to be associated with altered regulatory pathways for stathmin expression and function (38). These observations suggest that a potentially effective therapeutic approach could be designed that combines stathmin inhibition with pharmacologic agents that stabilize the mitotic spindle (39).

BRCA1. Breast cancer 1 gene (BRCA1) is a tumor suppressor gene implicated in predisposition to early onset of breast and ovarian cancer. Germline mutations in BRCA1 account for nearly half of hereditary breast cancer cases and most of breast/ovarian cancer cases. A role for BRCA1 in microtubule dynamics and mitosis is indicated by its centrosomal localization during mitosis. It is proposed that loss of BRCA1 ubiquitin ligase activity could cause centrosome hypertrophy and subsequent aneuploidy typically found in breast cancers. A definitive role for BRCA1 in microtubule nucleation is supported by the observation that BRCA1-dependent ubiquitination of γ-tubulin inhibits microtubule nucleation, and that a mutant BRCA1 protein, which lacks ubiquitin ligase activity, fails to inhibit MT nucleation (40).

Consistent with its role in microtubule nucleation during mitosis, BRCA1 seems to mediate sensitivity of breast cancer cells to apoptosis induced by microtubule-targeting agents. For example, BRCA1 mutant cell line H1CC1937 is more sensitive to the Vinca alkaloid vinorelbine than cell lines with wild-type BRCA1 gene (41). The role of BRCA1 in determining the sensitivity to taxanes is, however, not clear (42).

VHL tumor suppressor protein. von Hippel-Lindau disease is a heritable multisystem cancer syndrome that is associated with a germline mutation of the VHL tumor suppressor gene on the short arm of chromosome 3. Affected individuals are at risk of developing various benign and malignant tumors of the central nervous system, kidneys, adrenal glands, pancreas, and reproductive adnexal organs (43).

VHL protein (pVHL) is a MAP involved in regulation of microtubule dynamics and can protect microtubules from depolymerization in vivo (44). Both the microtubule binding and stabilization functions of pVHL are located within the mutational “hotspot” in VHL disease. Naturally occurring pVHL mutants that predispose to hemangioblastoma and phaeochromocytoma disrupt the microtubule-stabilizing function of pVHL. Thus, pVHL function in the regulation of microtubule dynamics potentially provides a link between defects in its expression and the pathogenesis of VHL syndromes (44).
Apo1ptosis Inhibitors

Survivin. Survivin is the smallest member of the inhibitory apoptosis protein family that is characterized by the presence of BIR domain (baculovirus inhibitory apoptosis protein repeat) that allows caspase inhibition (45). Survivin is overexpressed in tumors of almost all cell types (reviewed in ref. 46), whereas normal cells from these same organs do not express survivin (47).

Reduction or loss of survivin in mammalian cells has been associated with cell division defects that include supernumerary centrosomes, aberrant spindle assembly mislocalization of mitotic kinases (48), loss of mitotic checkpoint(s) (49), and cytokinesis failure with appearance of multinucleated cells. Survivin functions at cell division to control microtubule stability and assembly of a normal mitotic spindle. This pathway may facilitate checkpoint evasion and promote resistance to chemotherapy in cancer (50). Cells microinjected with the polyclonal antibody to survivin exhibit delayed progression in prometaphase and metaphase and display short mitotic spindles severely depleted of microtubules and occasionally undergo apoptosis without exiting the mitotic block or immediately after exiting mitotic block. Overexpression of survivin in cancer may overcome this apoptotic checkpoint and favor aberrant progression of transformed cells through mitosis (51).

Survivin plays a role in the mitotic response in the context of Taxol resistance. Taxol-resistant ovarian cancer cells exhibit defective mitotic response to the drug and fail to up-regulate survivin levels and survivin phosphorylation, in contrast to their parental drug-sensitive counterparts. Ectopic expression of wild-type survivin in Taxol-resistant cells restores their mitotic response to the drug. Studies on survivin have provided novel insights into the mechanisms of mitotic arrest and apoptosis induced by microtubule-targeting agents (52).

Microtubule Motor Proteins and Spindle Checkpoint Components

Microtubule motor proteins (kinesins) and centrosomal proteins known to be involved microtubule functions in mitosis are therefore potential targets for cancer therapy. Eg5 is critical for proper spindle formation during mitosis and it has received the most attention as a target for cancer therapy (53). Small molecule inhibitors of Eg5, such as monastrol, induce mitotic arrest and show antitumor activity (54). Inhibitors of Eg5 have been shown to be effective on both Taxol-sensitive and Taxol-resistant cells, which either have multidrug resistance product P-glycoprotein overexpression or acquired β-tubulin mutations. Interestingly, the combination of Eg5 inhibitors with Taxol produce an antagonistic effect on mitotic arrest and cell death, indicating that the combination of an Eg5 inhibitor with Taxol may not be of therapeutic use.

NudC protein associates with microtubule motor dynein/dynactin complex that regulates microtubule dynamics (55). Overexpression of NudC in LNCaP cells inhibits their anchorage-independent growth in a soft agar colony assay. Expression of NudC in DU145 or PC-3 cells inhibits tumor growth in a s.c. xenograft model, and NudC is a potential candidate for therapeutic approaches that target the function of a microtubule motors (56).

Expression of a kinesin-3 family member (KIF14) is associated with poor-prognosis breast cancer (57). However, due to the limited functional annotation for KIF14 and its Drosophila orthologues, it is difficult to speculate on how KIF14 contributes to breast cancer progression.

Drp1/Rit42, originally identified as a p53 target gene that is up-regulated in response to DNA-damaging agents, is a MAP that localizes to the centrosomes and participates in the spindle checkpoint in a p53-dependent manner. Rit42 seems to play a role in the regulation of microtubule dynamics and the maintenance of euploidy (58). Rit42 is known to be down-regulated during colon and prostate tumor progression (59, 60). Blocking endogenous Rit42 expression by small interfering RNA in normal human mammary epithelial cells results in the disappearance of astral microtubules and polyploidy.

Perspectives

It is now clear that the term MAP describes not only structural proteins thought to be found mainly in axons and dendrites of neurons but also a variety of proteins with important biological activities related to cancer predisposition and tumor formation and progression. Similar to two classes of chemical agents that alter microtubules, MAPs can either stabilize or destabilize microtubules. Therefore, variable expression of these proteins among different types of human malignancies and among individual patients with the same type of cancer has implication for outcomes in chemotherapy using microtubule-targeting agents. Although microtubule-targeting drugs are widely used for treatment of a several cancers, toxicity and tumor resistance have stimulated an intense search for more effective agents. A rational design of compounds that can bind (e.g., with higher affinity to Vinca, paclitaxel, or to other novel domains of tubulin based on crystal structure) is an avenue that merits further exploration. Experiments in vitro and multitude of correlative observations in vivo on the MAP expression and sensitivity of different cancers to microtubule-targeting drugs argue for adopting rational approaches that include screening for MAPs known to be expressed in individual cancers or, better yet, comprehensive proteomic profiling. Knowledge of tumor MAP profile and the available knowledge, however limited, on regulation of MAP expression could facilitate combination therapies with agents that produce a change in expression of relevant MAP in the desired direction (either up-regulation or down-regulation; Fig. 2). In addition to change in expression of MAPs, strategies to alter MAP-tubulin interaction can be explored. High-throughput screening of small molecule libraries is an attractive strategy to identify compounds that disrupt MAP-tubulin (e.g., stathmin-tubulin) interaction. Such compounds alone or in combination with microtubule-targeting drugs that are in use currently could offer more effective therapies.

Additionally, expression of “neuron-specific” MAPs, such as tau and MAP2, in nonneuronal cancers has implications for the origin and transdifferentiation of such cancers. For example, activation of neuronal MAPs in cancers (such as melanoma, breast cancer, prostate cancers, etc.) might reflect their origin from stem cell–like precursors. Alternatively, it may indicate plasticity of tumor cells to differentiate. Variable expression of MAPs and their effect on sensitivity to microtubule-targeting...
MAPs and resistance/sensitivity of cancer cells to the action of microtubule-targeting drugs. The action of chemotherapeutic agents that stabilize (red) or destabilize (green) microtubules is regulated by intracellular proteins (stabilizers, red and destabilizers, green) that influence microtubule dynamics. These proteins include tumor suppressors, oncogenes, and microtubule motor proteins. Disruption of microtubules by treatment with microtubule-targeting drugs triggers apoptosis by two mechanisms: in cells that are arrested in G2/M phase of cell cycle activation of G2-M checkpoint control leads to p53-independent cell death and in cells that escape G2-M checkpoint and progress through mitosis activation of G2-S checkpoint at subsequent rounds of the cell cycle leads p53-dependent apoptosis. However, cancer cells that are resistant to these agents could achieve this resistance by (1) activating drug efflux pump, (2) increase in expression of microtubule-destabilizing proteins in case of microtubule-stabilizing drugs, or (3) increase in expression of microtubule-stabilizing proteins in case of microtubule-destabilizing drugs. Pgp, P-glycoprotein.

drugs highlights, presumably, natural selection that favors preservation of microtubule dynamics, which is critical for rapidly dividing cells. Conversely, inappropriate expression of MAPs and disruption of microtubule dynamics, as in early cutaneous melanoma, could be a defensive mechanism to block runaway proliferation of oncogene-transformed cells similar to senescence and apoptosis. Therefore, understanding the molecular mechanisms that regulate expression of MAPs in cancers will not only open new avenues to increase the therapeutic efficacy of microtubule-targeting drugs but also shed new light on the role of this interesting family of proteins in the biology of cancer.

References


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