REVIEW ARTICLE

Mechanisms of Action of and Resistance to Antitubulin Agents: Microtubule Dynamics, Drug Transport, and Cell Death

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Purpose: **To analyze the available data concerning mechanisms of action of and mechanisms of resistance to the antitubulin agents, vinca alkaloids and taxanes, and more recently described compounds.**

Design: **We conducted a review of the literature on classic and recent antitubulin agents, focusing particularly on the relationships between antitubulin agents and their intracellular target, the soluble tubulin/microtubule complex.**

Results and Conclusion: **Although it is widely accepted that antitubulin agents block cell division by inhibition of the mitotic spindle, the mechanism of action of antitubulin agents on microtubules remains to be determined. The classic approach is that vinca alkaloids depolymerize microtubules, thereby increasing the soluble tubulin pool, whereas taxanes stabilize microtubules and increase the microtubular mass. More recent data suggest that both classes of agents have a similar mechanism of action, involving the inhibition of microtubule dynamics. These data suggest that vinca alkaloids and taxanes may act synergistically as antitumor agents and may be administered as combination chemotherapy in the clinic. However, enhanced myeloid and neurologic toxicity, as well as a strong dependence on the sequence of administration, presently exclude these combinations outside the context of clinical trials. Although the multidrug resistance phenotype mediated by Pgp appears to be an important mechanism of resistance to these agents, alterations of microtubule structure resulting in altered microtubule dynamics and/or altered binding of antitubulin agents may constitute a significant mechanism of drug resistance.**

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TUBULIN-BINDING AGENTS constitute a large family of compounds that have been used in a wide variety of ways, including as herbicides and antiparasitics and in human therapeutics. The first tubulin-binding agent to be used in humans was colchicine, extracted from *Colchicum autumnale*, which has been administered to patients with gout since sixth century AD.[1] The ability of colchicine to block cells in metaphase made it a powerful tool in the study of mitosis.[2] Tubulin, the building block of microtubules, was first identified as the ''colchicine-binding protein.''[3] The ability of some compounds to act electively on nonhuman cells, such as yeast, has been shown to be due to differences in these compounds' abilities to bind to human versus nonhuman tubulins. Despite structural constraints, significant variations in the primary structure of tubulin, as well as the emergence of various isotypes, have occurred during evolution.[4]

In the field of antineoplastic chemotherapy, tubulin-binding agents constitute an important class of compounds, with broad activity both in solid and in hematologic neoplasias.[5-11] These agents are believed to block cell division by interfering with the function of the mitotic spindle, blocking the cells at the metaphase/anaphase junction of mitosis.[12,13] Vinca alkaloids, the earliest tubulin-binding agents to be used in the clinic as antimitotics, have been described as ''microtubule depolymerizing agents.'' At high concentrations, these agents reduce or abolish the microtubule content of cells in culture and prevent polymerization of purified tubulin in vitro. Conversely, the taxanes paclitaxel and docetaxel promote the polymerization of purified tubulin in vitro and, at high concentrations, enhance the fraction of polymerized tubulin in cells and they have thus been referred to as ''microtubule stabilizing agents.''

MICROTUBULE STRUCTURE AND FUNCTION

Microtubules are composed of a backbone of tubulin dimers and microtubule-associated proteins (MAPs).[14] Alpha- and beta-tubulin peptides, both of which have molecular masses close to 50 kd, combine stoichiometrically to form tubulin dimers. Gamma-tubulin, which is less abundant, appears to be localized in the centrosomes.[15] Chaper-

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onins as well as proteins involved in tubulin folding appear to play an essential role in the synthesis of functional tubulin subunits.[16] Alpha- and beta-tubulins have been studied in many species, gamma-tubulin has been studied in a few, and sequence analyses have demonstrated strong conservation throughout evolution from yeast to human.[4] Alpha- and beta-tubulins exist under the form of isotypes, which are distinguished by slightly different amino acid sequences.[17,18]

Thanks to the work of Cowan et al[19-21] and Dobner et al,[22] six alpha- and six beta-tubulin isotypes have been described in mammals. The analysis of human tubulin genes has been complicated by the fact that many of the genes of the tubulin multigene family, identified by screening of genomic libraries, are in fact pseudogenes, which do not code for intact proteins.[23] The six mammalian beta-tubulin isotypes may be grouped into six classes, according to their C-terminal amino acid composition, which is the most highly divergent portion between isotypes, although they are highly conserved between species (Table 1). Posttranslational modifications have been reported, including phosphorylation and glutamylation (reviewed in Luduena[18]).

The strong intraspecies conservation of beta-tubulin isotypes has prompted a number of investigators to search for functional differences specific to the various isotypes. Analysis of tubulin isotype expression in various tissues has demonstrated a complex pattern of distribution, suggesting functional specificity. In neurons, there is evidence of isotype segregation within cells, as well as differential synthesis and phosphorylation during neurite outgrowth.[24] Conversely, immunohistochemical analyses of various microtubules (spindle, interphase, midbody, manchette, flagella) have failed to show segregation of isotypes into specialized microtubular structures, as have experiments with transfected tubulin isotypes.[25,26] The nature and degree of the functional specificities of beta-tubulin isotypes remain controversial.[18]

DYNAMICS AND FUNCTION

Microtubules are highly dynamic structures that are in unstable equilibrium with the pool of soluble tubulin dimers present in the cell. There is constant incorporation of free dimers into the polymerized structures and release of dimers into the soluble tubulin pool. Polymerization of tubulin dimers may be influenced by a number of factors, such as guanosine triphosphate, which binds to one exchangeable site on beta-tubulin and one nonexchangeable site on alpha-tubulin; the ionic environment; and MAPs. MAPs constitute a complex family of proteins, including MAP2, MAP4, Mip-90, tau, and STOP, many of which have been shown to regulate tubulin polymerization and function.[27-31] Many results have been reported on tubulin polymerization, with studies using highly purified tubulin, usually obtained from bovine brain, an abundant source. The development of real-time contrast videomicroscopy has allowed direct visualization of the behavior of individual microtubules.

Microtubule ends have the ability to switch stochastically between growing and shortening states, both in cells and in vitro. This phenomenon, called dynamic instability, is an essential property that makes microtubules some of the most plastic protein polymers in the cell.[32] Microtubules have a plus end, which is kinetically more dynamic than the other (the minus end). Although both ends alternately grow or shorten, net growing occurs at the plus end and net shortening at the minus end. When both of these actions occur simultaneously, the microtubule is said to be treadmilling, a phenomenon that is believed to be critical in the polar movement of chromosomes during anaphase.[33]

Microtubules are complex polymeric structures that are involved in a number of cellular functions.[3,14] They play a critical role not only in mitosis but also in intracellular transport, axonemal motility, and constitution of the cytoskeleton. The abundant amount of tubulin in neurons and the role of microtubules in axonal transport are thought to contribute to the neurologic toxicity of tubulin-binding agents in the clinic.[34] It is widely accepted that the antimitotic effect of the tubulin-binding agents used as anticancer agents is due to their effect on the mitotic spindle. However, these compounds also affect microtubules in interphase cells, altering neurite morphogenesis, as well as adhesion and locomotion properties.[35-37] Other antitumor effects of taxanes have been

Table 1. Beta-Tubulin Isotypes in Vertebrates

Class	Isotype			% Homology (mouse/ human)	C-Terminal Sequence	Expression
	Human	Chicken	Mouse			
I	M40	cβ7	mβ5	100	EEEEDFGEEAEEEA	All tissues
II	hβ9	cβ1/cβ2	mβ2	100	DEQGEFEEEGEEDEA	Major: neuronal, many tissues
III	hβ4	cβ4	mβ6	99	EEEGEMYEDDEEESESQGPK	Minor: neuronal
IVa	h5β	—	mβ4	100	EEGEFEEEAEEEVA	Major: neuronal
IVb	hβ2	cβ3	mβ3	100	EEGEFEEEAEEEVA	Major: testis, many tissues
V	ND	cβ5	ND	—	NDGEEEAFEDDEEEINE	All tissues except in neurons
VI	hβ1	cβ6	mβ1	91	EEDEEVTEEAEMEPEDKGH	Hematopoietic specific

Abbreviation: ND, not described in this species.

described that appear to be independent of the antimitotic activity. Paclitaxel modifies the motility of paclitaxel-resistant ovarian carcinoma cells in vitro and displays antiangiogenic activity in vivo.[38,39] The specific action of tubulin-binding agents on the mitotic spindle may be attributed to the fact that mitotic microtubules are considerably more dynamic than interphase microtubules, with a much shorter half-life.[40] Conversely, the absolute requirement of a functional spindle for the proper migration of chromosomes during anaphase may explain why this stage of the cell cycle is particularly vulnerable to tubulin active agents, even though these compounds act on other cellular microtubules as well.[41]

HOW TUBULIN-BINDING AGENTS WORK

Despite considerable efforts, the exact binding sites of tubulin-binding agents on microtubules have not been identified. However, Nogales et al[42] recently presented the results of a crystallographic analysis that defined the paclitaxel binding site more precisely. Although it has been shown that tubulin dimers are the targets of these compounds, whether the beta-tubulin subunit is the exclusive binding site for these compounds has not been clearly determined.[1,43,44] Although evaluation of total accumulation of labeled compounds in cells is technically straightforward, quantification of drug binding to microtubules is more difficult. Cells displaying the multidrug resistance (MDR) phenotype have a reduced amount of total drug, because of increased drug efflux. However, to date, there are no reports describing a specific association between resistance to tubulin-binding agents and reduced drug binding to microtubules.

Colchicine and vinca alkaloids exert their effects on microtubules under different conditions. Unlike vinca alkaloids, colchicine must first bind to soluble tubulin before acting on microtubule dynamics. At substoichiometric concentrations ($<$ one molecule of drug for each molecule of tubulin), these compounds dramatically affect microtubule dynamics, without causing depolymerization.[32] It is believed that tubulin-colchicine and tubulin–vinca alkaloid complexes, and unbound vincas, bind to and ''poison'' microtubule ends, changing both on- and off-rate constants, thereby considerably reducing their ability to grow or shorten.[45] At higher concentrations, these compounds bind stoichiometrically to tubulin subunits and can induce rapid polymer disassembly, giving rise to nonmicrotubular structures such as vincristine-induced spiral protofilaments. The net effect of these high concentrations is a reduction or a disappearance of the normal microtubule network of the cell.

Taxanes, on the other hand, bind to polymerized tubulin only.[46] There is a binding site for paclitaxel on each tubulin dimer in microtubules, and the ability of paclitaxel to induce polymerization is associated with stoichiometric binding of paclitaxel to microtubules. However, at lower, substoichiometric, concentrations (one molecule of paclitaxel for 200 to 600 molecules of tubulin), paclitaxel suppresses microtubule dynamics without significantly altering the microtubule polymer mass.[47,48] Paclitaxel also modifies the rigidity of microtubules, an effect that may contribute significantly to its effect on mitosis.[49] Thus, at very low concentrations, all of these compounds share the ability to reduce microtubule dynamics while not significantly affecting the amount of polymerized tubulin.

Attempts have been made to correlate the isotype composition of microtubules with their dynamic properties and/or their different abilities to bind tubulin-binding agents. Luduena et al[50] reported that colchicine binding was biphasic in preparations of bovine brain tubulin, which is a mix of classes I, II, III, and IV, but monophasic in the case of renal tubulin, which does not contain class III beta-tubulin. Falconer et al[51] showed that colchicine-stable microtubules preferentially incorporate class II beta-tubulin. Lobert et al[52] reported that the interaction of vinblastine with tubulin is identical for all beta-tubulin isotypes but that class III beta-tubulin differs from unfractionated tubulin in its ability to associate into paclitaxel-stabilized microtubules. Laferriere and Brown[53] found that paclitaxel promoted the polymerization and posttranslational modifications of class III beta-tubulin in an embryonal carcinoma cell line. Panda et al[54] reported that immunopurified isotypes of tubulin display different assembly properties in vitro. Derry et al[55] showed that paclitaxel differentially modulates the dynamics of microtubules assembled from unfractionated and purified beta-tubulin isotypes.

Taken together, these data suggest that tubulin isotypes may be important determinants of microtubule dynamics. These results, as well as those showing altered tubulin isotype content in resistant cell lines, suggest that the isotype composition of microtubules may influence sensitivity to tubulin-active agents. However, the tubulin isotype profile of mammalian cells is complex and is variable from one tissue to another. At present, no simple relationship has been established between the level of expression of a given tubulin isotype and the degree of sensitivity or resistance to a given tubulin-binding agent.

MECHANISMS OF RESISTANCE TO ANTITUBULIN DRUG TRANSPORT

At present, the best described mechanism of resistance to tubulin-binding agents is the MDR phenotype, mediated by the 170-kd Pgp efflux pump, encoded by the *mdr*1 gene.[56,57]

Both the vinca alkaloids and the taxanes are good substrates for this pump.[58,59] In a number of cases, development of cell lines resistant to vincristine or paclitaxel has been shown to be associated with the expression of *mdr*1.[57,60] The multidrug resistance protein has also been shown to be an efficient transporter of vinca alkaloids, but not taxanes.[61,62] Presently, little is known concerning the significance of the MDR phenotype in the emergence of resistant tumors in patients treated with tubulin-binding agents. Clinical trials aiming to sensitize MDR-positive tumors to agents such as vinblastine with Pgp modulators have been disappointing.[63]

Altered metabolism and/or subcellular distribution, alterations of the interaction between drugs and their target (microtubules), and altered response to cell cycle arrest induced by mitotic blockage are among the possible non-MDR mechanisms of resistance to tubulin-binding agents (Fig 1). To date, there have been no reports of cell lines that are resistant to tubulin-binding agents because of altered metabolism of these compounds. Regulation of glutathione levels by buthionine sulfoximine has been reported to influence paclitaxel-induced cytotoxicity, but it is not clear whether this is due to an effect on drug metabolism or to a direct interaction between glutathione and tubulin.[64,65]

MICROTUBULE DYNAMICS AND RESISTANCE TO TUBULIN-BINDING AGENTS

Cabral et al[66-68] described a model in which resistance to tubulin-binding agents is associated with the presence of alterations in microtubule stability. According to these authors, some cells contain ''hypostable'' microtubules, with a spontaneous tendency toward depolymerization, and ''hyperstable'' microtubules, with a relative resistance to depolymerization. In this model, cells with hypostable microtubules are particularly susceptible to the depolymerizing agents and display hypersensitivity to vinca alkaloids while displaying resistance to the stabilizing agents (Fig 2). Conversely, cells containing hyperstable microtubules are resistant to the vinca alkaloids but relatively sensitive to the taxanes. This model offers an explanation for the phenomenon of paclitaxel-dependent cell lines, in which cells do not grow in the absence of paclitaxel.[69] According to this model, the dependence on paclitaxel is due to the presence of extremely hypostable microtubules that, in the absence of a stabilizing agent, disassemble spontaneously and are incompatible with normal cell function.

Using clinically relevant concentrations of vinblastine and paclitaxel, Jordan et al[12,13,70] showed that both depolymerizing and stabilizing agents exert antimitotic effects by reducing spindle microtubule dynamics, with no significant alteration in the distribution of tubulin between the soluble and the polymerized forms. Using real-time differential-interference contrast videomicroscopy, these authors analyzed the dynamic behavior of individual microtubules and found that vinblastine strongly reduces microtubule dynamics, without significantly modifying the length of the microtubules (or absolute microtubular mass). Analyzing the effects of paclitaxel at low concentrations, these authors found the same effect on microtubule dynamics, with no significant alteration in microtubule length. In terms of the interactions of tubulin-binding agents with microtubules, the most meaningful equilibrium to consider may therefore be between highly dynamic microtubules and less dynamic

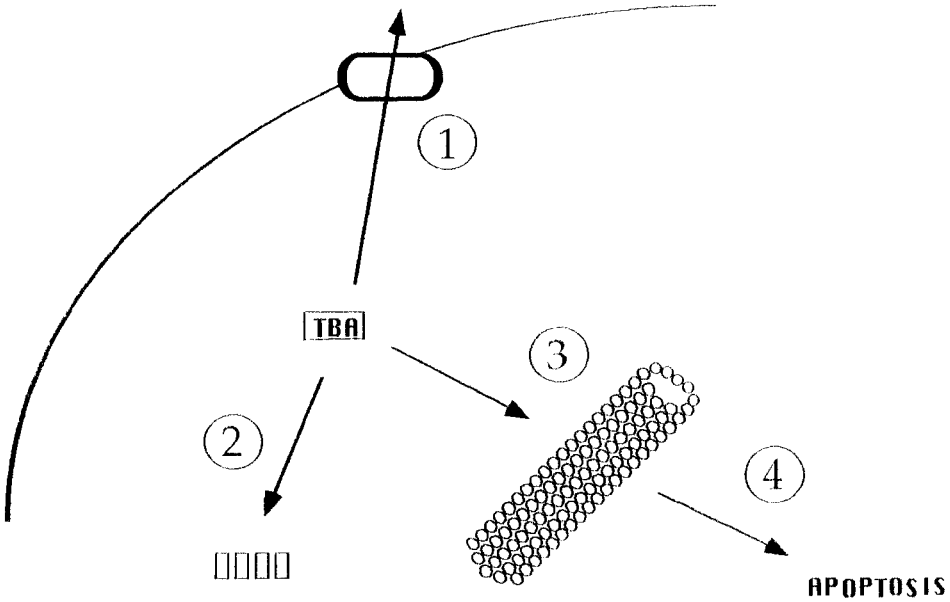

Fig 1. Potential mechanisms of resistance to tubulin-binding agents (TBA). 1: Efflux of drug by a membrane pump. 2: Altered metabolism or distribution of agent. 3: Altered interaction of agent with microtubules. 4: Inadequate induction of apoptotic signal.

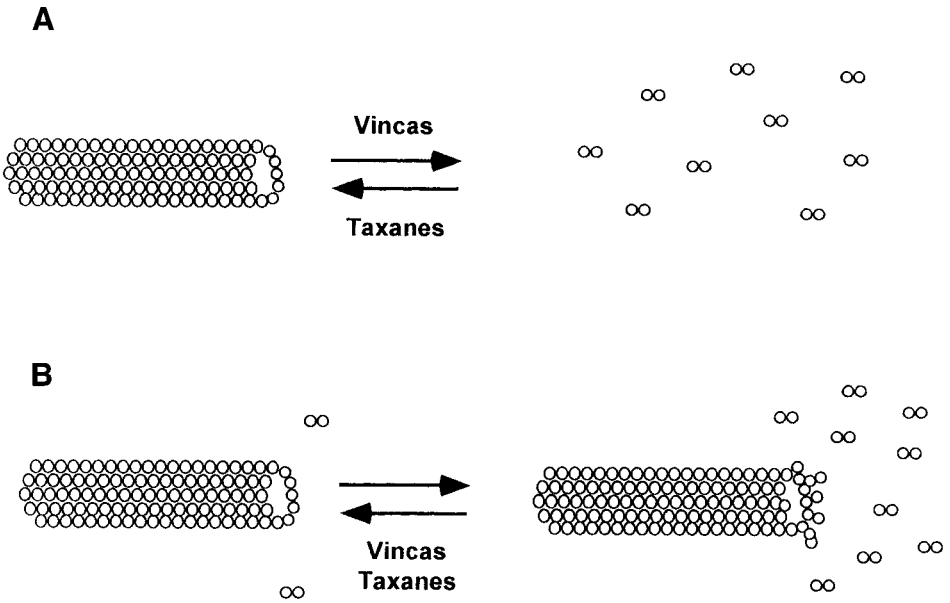

Fig 2. Models describing effects of tubulin-binding agents on soluble tubulin / microtubule complex. (A) Cabral model: Equilibrium between soluble tubulin dimers and polymerized tubulin (microtubules). Hypostable microtubules are sensitive to vinca alkaloids, hyperstable microtubules to taxanes. (B) Jordan and Wilson model: Equilibrium between highly dynamic microtubules and weakly dynamic microtubules. Binding of a drug to microtubules reduces or suppresses dynamics of highly dynamic microtubules, forming stabilized microtubules.

microtubules, rather than between polymerized and soluble tubulins (Fig 2).

These two models differ significantly in their prediction of cross-resistance to the vinca alkaloids and the taxanes. The Cabral model suggests that cells resistant to depolymerizing agents may be sensitive to stabilizing agents and vice versa. Conversely, in the Jordan and Wilson model, these two types of compounds exert the same suppressive effects on microtubule dynamics, and cells resistant to one class of compounds may thus be cross-resistant to the other, at least in terms of interaction with the intracellular target. However, the concentrations involved in the two models differ greatly, and the net effect on microtubule polymerization, a critical parameter in the Cabral model, probably occurs only at high concentrations of drugs. These high concentrations, which may allow stoichiometric interaction between the tubulin-binding agents and tubulin, may be difficult or impossible to achieve clinically.

There is a growing body of evidence suggesting that some combinations of vinca alkaloids and taxanes may be beneficial in terms of antitumor activity. Aoe et al[71] reported synergy between vinorelbine and docetaxel on a human lung cancer cell line in vitro, and Photiou et al[72] showed synergy between paclitaxel and vinorelbine against human melanoma lines. In the P388 murine model, Knick et al[73] reported not only a significant percentage of long-term cures with the combination of vinorelbine and paclitaxel, but also a reduced toxicity of these agents when they were used in combination. Of note is the importance of the delay between the administration of these two agents: the same doses were lethal to 80% of the animals when administered 24 hours apart but well tolerated when administered less than 6 hours apart. Preliminary reports of combinations of vinorelbine with paclitaxel or docetaxel in patients with advanced breast cancer or lung cancer suggest promising activity with no substantial increase in toxicity.[74-76] Conversely, Monnier et al,[77] who studied the effects of the combination of docetaxel and vinorelbine in 26 chemotherapy-naive patients with non–small-cell lung carcinoma, reported substantial hematologic and mucosal toxicity, with two toxic deaths, and studies of paclitaxel-vinorelbine combinations showed severe and/or frequent neurotoxicity.[78,79] Additional clinical data are clearly required to evaluate the benefit of the combination of vinca alkaloids and taxanes, and such combinations should not be administered outside prospective clinical trials.

TUBULIN GENES AND DRUG RESISTANCE

The available data suggest that alterations in microtubule structure and/or function represent an important, and potentially complex, mechanism of resistance to tubulin-binding agents. A number of cell lines resistant to tubulin-binding agents in vitro have been shown to contain tubulin alterations, in terms of total tubulin content, tubulin polymerization, or tubulin isotype content.[80-82] We reported that the KPTA5 cell line, which is exclusively resistant to taxanes, displays increased expression of the class IVa tubulin isotype.[82] Conversely, the KCVB2 cell line, which does not express *mdr*1, is cross-resistant to vinca alkaloids and to taxanes and has a reduced amount of total tubulin, a higher percentage of polymerized tubulin, and a higher content of class III tubulin isotype.[83] Various investigators have re-

ported altered expression of tubulin isotypes in resistant cell lines.[81,84,85] Haber et al[86] reported that in the murine cell line J774, resistance to paclitaxel is associated with a 21-fold increase in class II beta-tubulin isotype. In paclitaxel-resistant human prostate cancer cells, on the other hand, class III beta-tubulin appears to be overexpressed.[87] Mutations of tubulin isotype genes have also been reported in paclitaxel-resistant lines.[88] Reproducing resistant phenotypes by modifying the tubulin isotype composition of cells has proven to be difficult[89] and has been impeded due to the fact that there are often multiple alterations of the soluble tubulin/microtubule complex in resistant lines.

PROGRAMMED CELL DEATH (APOPTOSIS)

Tubulin-binding agents induce apoptosis in tumor cells in vitro, as do a great number of other chemotherapeutic agents.[90] The mechanism by which mitotic blockage induces apoptosis remains to be determined, although it is increasingly clear that a number of regulatory molecules,[91,92] as well as oncogenes,[93] bind to the mitotic apparatus. It is highly probable, although the mechanism is poorly understood, that genes that protect cells against apoptosis, such as mutant *p53*, *bcl-2*, and *bcl-x*, may induce resistance to tubulin-binding agents.[94,95] MAPs are also likely to be involved in mechanisms of resistance to drug-induced apoptosis. MAP4, the expression of which is negatively regulated by wild-type *p53*, has been shown to increase sensitivity to paclitaxel.[96,97] Tau overexpression has been described in estramustine-resistant human prostatic carcinoma cells.[98]

The relationship between *p53* alterations and sensitivity to antitubulin agents is complex. Functional *p53* causes cell cycle arrest in the G1 phase in case of DNA damage, thereby allowing DNA repair and enhanced survival in normal cells. It was thus expected that abnormal *p53* would sensitize tumor cells to DNA-damaging agents. In most cases, however, abnormal *p53* was associated with drug resistance. These unexpected findings were attributed to the fact that tumor cells that did not express functional *p53* were unable to initiate apoptosis because of the DNA damage they had sustained. The temporary inactivation of *p53* by acute human papillomavirus or the permanent inactivation obtained in *p53*-null mice is associated with increased sensitivity to paclitaxel.[99,100] Woods et al[101] suggested that paclitaxel induces apoptosis through two different pathways: a *p53*-independent pathway occurring in cells blocked in prophase, which is observed both in *p53*-expressing and in *p53*-null mouse embryo fibroblasts; and a *p53*-dependent mechanism, which occurs in cells that accumulate in G1 and requires functional *p53*. The observation by various authors that vinca alkaloids and paclitaxel induce *p53* may thus be interpreted as a resistance mechanism of the cell against the cytotoxic effect of paclitaxel.[102,103] Paclitaxel has been shown to modulate the level of expression of genes involved in apoptotic regulation, such as *bcl-x*$_L$.[104] The ability to regulate gene expression appears to be an important property of paclitaxel but not of docetaxel.[105]

NEW TUBULIN-BINDING AGENTS

New antitubulin agents are currently being evaluated (Table 2). Spurred by the encouraging results obtained with taxanes, research has continued, yielding alkylating paclitaxels that bind irreversibly to tubulin and are active at lower concentrations on tumor cell lines.[106] Nontaxane stabilizing agents have also been described. Estramustine suppresses microtubule dynamics and displays synergism with vinblastine.[107,108] Discodermolide, extracted from the Caribbean sponge *Discodermia dissoluta*, stabilizes microtubules more potently than paclitaxel and inhibits the growth of breast cancer cell lines in vitro.[109,110] The macrolides epothilones A and B also share the ability to arrest cells in mitosis and promote the formation of microtubular bundles in nonmitotic cells.[111,112] A number of peptide agents have been shown to block cell division by interfering with microtubule function. These include dolastatin and cryptophycin, which behave as depolymerizing agents and inhibit the binding of vinblastine to tubulin.[113-115] Cryptophycin induced more prolonged depletion of microtubules in vitro than did

Table 2. New Antitubulin Agents

Compound	Origin	Competes With	Range of Activity	Sensitivity to MDR	Comments
Discodermolide	Sponge (*Discodermia dissoluta*)	Taxanes	< nM	Low	Possibly immunosuppressive, more potent than paclitaxel
Epothilones A and B	Myxobacterium (*Sorangium cellulosum*)	Taxanes	nM	Low	No endotoxin-like effect; equipotent with paclitaxel
Dolastatin	Mollusk (*Dolabella auricularia*)	Vincas	μM	High	Peptide
Cryptophycins	Cyanobacterium	Vincas	pM	Low	Peptide; active in murine models
Curacin A	Cyanobacterium (*Lyngbya majuscula*)	Colchicine	nM	NA	Thiazoline ring–containing lipid

Abbreviation: NA, not available.

vinblastine.[116] Many of these new compounds, with the exception of dolastatin, are weakly transported in Pgp-expressing cells and thus retain activity in cells expressing the MDR phenotype. Although the first tubulin-binding agents have been extracted from plants and trees, most of the recent and promising compounds have been found in marine organisms.

In conclusion, tubulin-binding agents constitute a diverse group of compounds with many applications in medicine. Cytotoxic tubulin-binding agents are unique among anticancer drugs in that they target the mitotic spindle rather than DNA. Although vincas and taxanes may differ in their gross effect on cellular cytoskeleton in culture, these compounds seem to share a common mechanism of action—namely, the inhibition of microtubule dynamics. An important consequence is that the understanding, and possibly the therapeutic modulation, of factors influencing microtubular dynamics will be essential to improve the therapeutic efficacy of these compounds. Because of the high tubulin content in neuronal tissues, these agents also share a common side effect: neurotoxicity. The discovery of new marine compounds that are not MDR substrates offers great hope for the expansion of the role of this family of agents in the treatment of cancer.

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