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Discovering novel strategies for antimicrotubule cytotoxic therapy

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1. Introduction

Chemotherapeutic agents that target tubulin continue to be important components of current cancer therapy. To date, taxanes and vinca alkaloids have been the most clinically important classes of tubulin-binding drugs. Both are natural compounds that are highly effective in the treatment of a number of cancers because of their ability to disrupt microtubule dynamics by inducing a potent mitotic block and subsequent cell death.[1] Nonetheless, certain cancers either do not respond to treatment or develop resistance, which poses a major clinical problem for the use of these agents.

Considerable effort has been directed towards isolating and synthesising new antimitotic agents that target the microtubule system and display efficacy against drug-refractory carcinomas. Newly described compounds include natural products (e.g., dolastatin, discodermolide and epothilones), derivatives and structural analogues of traditional antimitotic agents, and novel synthetic molecules.

Recent research has also been directed towards deciphering the mechanisms by which cancer cells resist the effects of tubulin-targeted drugs, including alterations in the drug target (e.g., tubulin mutations), tubulin isotype expression and microtubule dynamics, and also modifications in microtubule-regulatory proteins; thus, providing additional opportunities for the development of novel therapies. This article describes the tubulin/microtubule system, with specific emphasis on mechanisms mediating resistance, and reviews strategies for enhancing the efficacy of microtubule-targeted cytotoxic therapy.

2. Tubulin and the microtubule system

Microtubules are composed of repeating α/β-tubulin heterodimers that self-associate into polymers.[2] Tubulin exists in different isotypic forms, and multiple isotypes (7α-tubulin and 7β-tubulin isotypes) with tissue-specific expression have been identified in human cells.[3] The various β-tubulin isotypes are evolutionarily conserved across species and differ from each other predominantly in their carboxy terminal region.[2] This region binds distinct microtubule-associated proteins (MAPs) and is therefore thought to influence microtubule stability and functionality. Tubulin isotype composition appears to be important for the cellular response to tubulin-targeting anticancer drugs.[2,3]

Microtubules are highly dynamic structures that play an integral role in cellular growth, vesicular transport and mitosis.[2] The ability of microtubules to polymerise and

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depolymerise is essential for the segregation of chromosomes during mitosis.[3] In an interphase cell, microtubules originate from the centrosome forming a hub and spoke-type network and are responsible for transporting substances around the cell. During cell division, this network is completely disassembled and reformed into mitotic spindles, across which duplicate sets of chromosomes line up and are divided equally into two daughter cells. Upon completion of mitosis, the spindle disassembles and the interphase microtubule network reforms. Factors that disrupt this process or affect microtubule dynamics will induce mitotic arrest and kill the cell. Not surprisingly, the tubulin/microtubule system is an important target for anticancer drug design.[1]

3. Effects of tubulin-binding drugs on microtubule structure and dynamics

Under normal circumstances, tubulin dimers and the microtubule polymer exist in a state of dynamic equilibrium within the cell (Fig. 1).[1] The addition of a destabilising agent, such as a vinca alkaloid (e.g., vincristine or vinblastine), at high concentrations (i.e., excess drug per tubulin molecule) pushes the equilibrium towards the tubulin dimers, resulting in disruption of the microtubule architecture. By contrast, the addition of a microtubule-stabilising agent, such as a taxane (paclitaxel or docetaxel), at high cellular concentrations enhances tubulin polymerisation, resulting in an increase in polymer mass and the formation of microtubule bundles.[1]

Although vinca alkaloids and taxanes disrupt cells in different ways, both classes of drugs bind to β-tubulin, either predominantly to the tubulin dimer (in the case of vinca alkaloids) or primarily to the microtubule polymer itself (in the case of taxanes).[1]

In drug-sensitive cells, tubulin-targeting agents are thought to disrupt microtubule dynamics by interfering with mitotic spindle function, inhibiting cell proliferation at the metaphase/anaphase junction of mitosis (Fig. 2).[4,5] Disruption of microtubule dynamics results in the failure of bipolar spindle attachment to the kinetochores of metaphase chromosomes.[6] This engages the spindle assembly checkpoint, which prevents the metaphase-to-anaphase transition.[6] Tumour cells then undergo cell-cycle arrest during the G_2/M phase of the cell cycle, followed by apoptotic cell death.[7] By contrast, resistance to tubulin-targeting drugs can result in cell survival and multiplication.[8] Mechanisms responsible for drug resistance are multifaceted and include those associated with the multidrug-resistance (MDR) phenotype and alterations in the microtubule and associated proteins.

Strategies to increase the effectiveness of antimicrotubule cytotoxic therapy in the management of cancer include the development of novel agents, the application of changes in tumour sensitivity following microtubule alteration to make treatment with existing agents more effective, and the down-regulation of specific β-tubulin subclasses or of regulators of microtubule function.

4. Novel antimicrotubule drugs

Several novel antimitotic agents have been identified and are being evaluated in clinical trials (Table 1). Dolastatin, cryptophycins and curacin A are microtubule-destabilising agents, whereas discodermolide and epothilones stabilise microtubules.[8] Both discodermolide and the epothilones are of particular interest in novel drug development because they inhibit growth of MDR cell lines.[9]

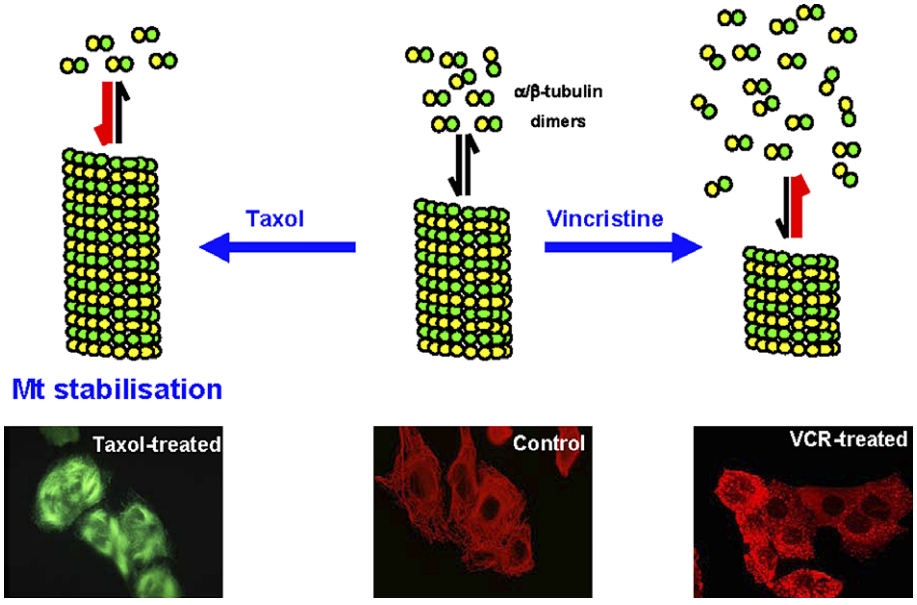

Fig. 1 – Microtubule stability reflects a dynamic equilibrium between tubulin dimers and the microtubule polymer. Stabilising agents such as the taxanes move the equilibrium towards the polymerised state, while destabilising agents such as the vinca alkaloids move it in the opposite direction. Reproduced, with permission, from Ref. [1] (reproduced with permission from Bentham Science Publishers Ltd).

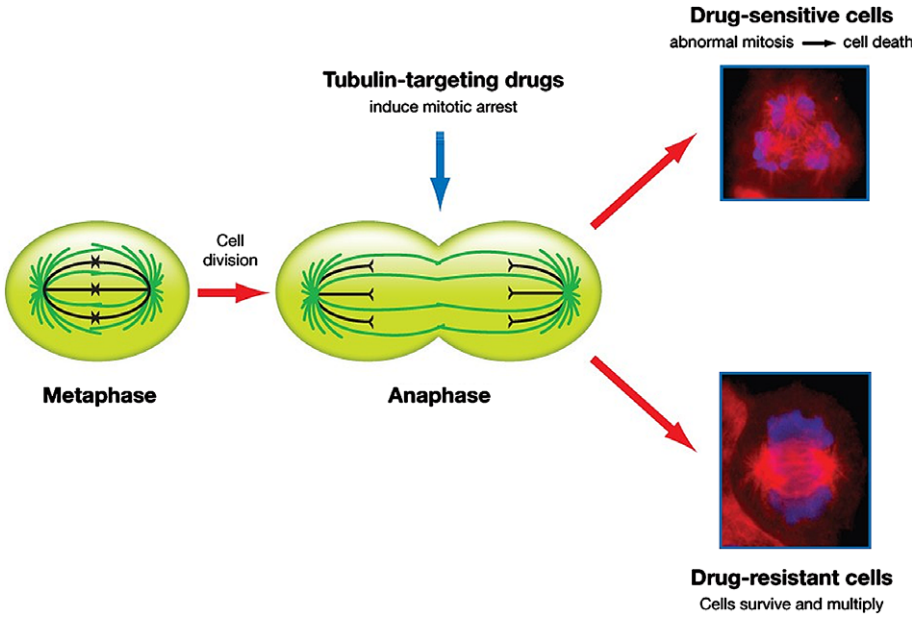

Fig. 2 – Effects of tubulin-targeting drugs on cancer cells.

Table 1 – Novel microtubule-targeted agents currently under evaluation[a]

Compound/substrate	Origin	Competes	MDR
Discodermolide	Sponge	Taxanes	Low
Epothilones Ixabepilone	Myxobacterium	Taxanes	Low
Dolastatin Dolastatin-10 TXT-1027	Marine mollusk	Vinca alkaloids	High
Cryptophycins Cryptophycin 52 and 55	Cyanobacterium	Vinca alkaloids	Low
Curacin A	Cyanobacterium	Colchicine	NA

MDR, multidrug resistance; NA, not applicable.
a Derivatives and structural analogues of vinca alkaloids and taxanes have displayed less cross-resistance to the MDR phenotype.

4.1. *Dolastatin*

Dolastatin-10 is a novel pentapeptide that inhibits microtubule assembly and tubulin polymerisation. In phase II studies, dolastatin-10 lacked significant clinical activity when used as a single agent in the treatment of hormone-refractory prostate cancer,[10] advanced breast cancer,[11] advanced pancreaticobiliary cancers[12] and treatment-naive patients with advanced melanoma.[13] Newly identified derivatives of dolastatin-10 have shown superior activity in vitro and in vivo in preclinical trials[14,15] and are currently being tested in phase I trials.[16,17]

4.2. *Cryptophycins*

Cryptophycins are active in breast and ovarian tumour cells that overexpress P glycoprotein and have shown promise in combination therapy with 5-fluorouracil, doxorubicin, paclitaxel and irinotecan.[18–20] Cryptophycin 52 is currently in early clinical development for the treatment of solid tumours.

4.3. *Curacin A*

Curacin A is a potent competitive inhibitor of colchicine binding to tubulin and causes a high level of growth inhibition in cancer cell lines. However, the low water solubility and lack of chemical stability of curacin A are significant detriments to its clinical development.[21]

4.4. *Discodermolide*

Discodermolide stabilises microtubules, inducing cell-cycle arrest during the G_2/M phase of the cell cycle. In vivo, it affects microtubule assembly more rapidly than paclitaxel.[9] It is significantly cytotoxic in P-glycoprotein-expressing cells, although not to the extent of epothilones.[9,22] Discodermolide

acts synergistically with paclitaxel and, unlike epothilones, it cannot be substituted for paclitaxel in a paclitaxel-dependent cell line.[22] Limited natural supplies have hampered in vivo studies, although large-scale synthesis has recently been achieved and the synthetic material is now undergoing phase I clinical trials for use in the treatment of pancreatic cancer.[23]

4.5. *Epothilones*

Epothilones were the first novel structural class of compounds found to have a taxane-like mechanism of action,[24] and they are the best studied of the new antimicrotubule agents. Data from structure–activity relationship studies suggest a common pharmacophore for binding of epothilones and taxanes.[25,26] Epothilones are competitive inhibitors of [^{3}H]-paclitaxel binding to microtubule polymers.[24,26,27] These data, combined with those from mutational and pharmacophore analyses, suggested an overlapping of binding sites for epothilones and paclitaxel.[26,28,29] Although it now appears that paclitaxel and epothilone do not have a common pharmacophore and that each ligand binds to the tubulin-binding pocket in a unique way.[30] Importantly, unlike paclitaxel, epothilones are not transported by P glycoprotein, thus, they retain their potency in MDR cell lines. Furthermore, epothilones have excellent cytotoxicity profiles in several paclitaxel-resistant cell lines containing β-tubulin mutations.[28] Epothilone analogues have shown efficacy against a range of tumour types, and a semi-synthetic analogue of epothilone B, ixabepilone [BMS-247550], is currently undergoing phase II/III clinical trials.[31]

Data on ixabepilone have been promising so far. In a clinical trial in 37 patients with metastatic or locally advanced breast cancer in whom neoadjuvant or adjuvant therapy with a taxane had previously failed, treatment with ixabepilone resulted in complete remission in 1 patient, partial remission in 7 patients and stable disease in 13 patients.[31]

5. Antimicrotubule alterations associated with antimicrotubule resistance

Despite the promise of novel tubulin-binding agents in the treatment of taxane-refractory tumours, there are probably some overlapping mechanisms of drug resistance, and a better understanding of what causes such resistance is needed if therapy is to be improved. Determining the mechanisms that mediate drug resistance has become an important area of research.[1] Several mechanisms may be involved in mediating resistance to antimicrotubule drugs, including drug efflux and processes that resist apoptosis. In this review, however, the focus is on mechanisms affecting the target of such drugs, namely tubulin mutations and altered expression of β-tubulin isotypes and microtubule-regulatory proteins (Fig. 3).[1]

5.1. *Tubulin mutations*

Mutations in the cellular target of tubulin-binding agents (i.e., the β-tubulin subunit) account for some of the observed resistance to these agents.[32] Several β-tubulin mutations have been identified using antimicrotubule-resistant cell lines (reviewed by Drukman and Kavallaris[32]). These mutations have varied effects on drug binding and microtubule stability.[32,33]

The consequences of tubulin mutations have been investigated by selecting for resistance-conferring mutations in lymphoblastic leukaemia cell lines through exposure to escalating concentrations of desoxyepothilone B (dEpoB), an epothilone analogue.[34] This resulted in mutations that push the microtubule equilibrium towards less-stable microtubules, making it more difficult for a microtubule-stabilising

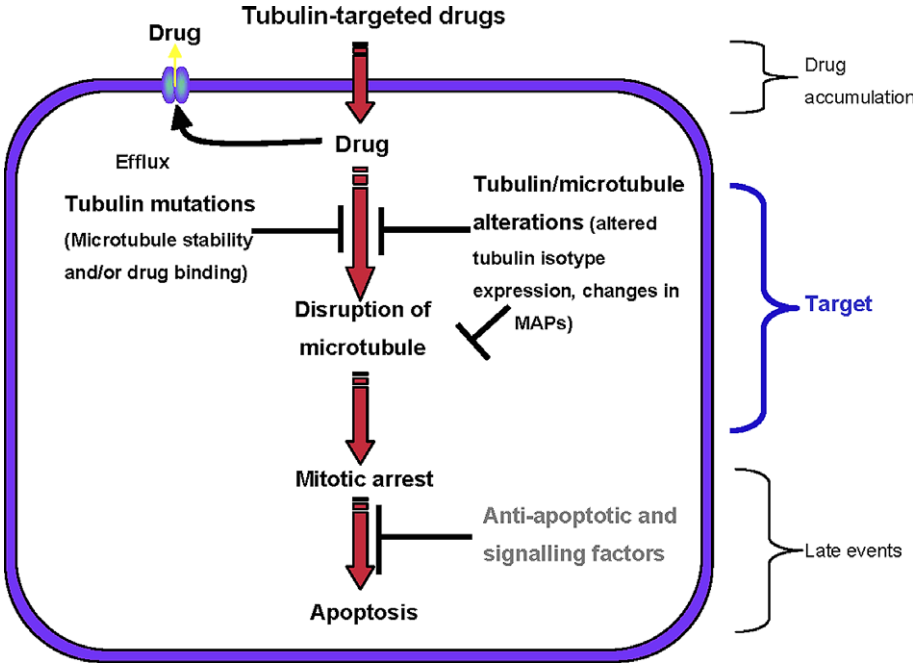

Fig. 3 – The mechanisms mediating resistance to antimicrotubule agents include drug efflux and anti-apoptosis as well those relating to the drug target. Reproduced, with permission, from Ref. [1] (reproduced with permission from Bentham Science Publishers Ltd).

drug to be effective. Importantly, the mutations produced cell lines that were hypersensitive to vinblastine, a destabilising agent, suggesting that an understanding of how the stability of the microtubules affects drug action could potentially direct therapy to patients who are more likely to respond.

5.2. *Changes in the expression of β-tubulin isotypes*

An increasing body of evidence suggests that the expression of specific β-tubulin isotypes is altered in cells that are resistant to antimicrotubule agents.[3,32,33] In particular, several studies have shown that poor response to taxane treatment is associated with increased expression of class III β-tubulin.[35–38]

In the first study that reported an association between β-tubulin isotype gene expression and taxane-resistant patient tumours, classes III and IVa β-tubulin were significantly increased in clinically derived paclitaxel-resistant ovarian epithelial tumour cells compared with untreated primary tumours.[35]

In subsequent research, Rosell et al.[36] showed that patients with non-small-cell lung cancer tumours expressing higher intrinsic levels of class III β-tubulin had shorter survival times when treated with paclitaxel and carboplatin therapy than those with tumours expressing low β-tubulin levels. In addition, increased expression of class III β-tubulin was associated with docetaxel resistance in human breast cancer.[38]

Downregulating class III tubulin expression has also been investigated as a way to restore taxane sensitivity in lung cancer cell lines.[39] In the first study of its type, paclitaxel-resistant lung cancer cells that displayed a 4-fold increase in Hβ4 expression compared with parental cells were treated with antisense phosphorothioate oligodeoxynucleotides. Results showed a concentration-dependent reduction in Hβ4 mRNA expression. Immunofluorescence staining showed a decrease in class III protein expression that corresponded to a significant increase in sensitivity to paclitaxel.[39] These results could be potentially clinically relevant if manipulation of the expression of specific β-tubulin isotypes and sensitisation of cancer cells to antimitotic agents, particularly stabilising agents, become possible. Future research might also enable the identification of small molecule inhibitors of these specific isotypes, which may be useful in combination therapies.

In contrast to changes in isotype expression, mutations in β-tubulin have not yet been associated with antimicrotubule drug resistance in ovarian, breast or lung cancer. It will be interesting to see how epothilones and their analogues progress in this regard and whether patients develop resistance and if so whether this is mediated by mutations in β-tubulin. Although mutations and mutational studies have been valuable in providing a better understanding of how drugs target the β-tubulin molecules, they do not appear to be relevant in terms of clinical studies.

5.3. *Altered expression of microtubule-regulatory proteins*

MAPs are important structural and regulatory components of microtubules. On the one hand, alterations in their expression can affect microtubule function and have been associated with resistance to antimicrotubule drugs. On the other hand, by manipulating the expression of microtubule proteins it is possible to enhance the action of antimicrotubule drugs. Increased expression of MAP4 for example has been associated with an increase in polymerised molecules, enhanced binding of paclitaxel, increased sensitivity to paclitaxel, and increased resistance to microtubule-destabilising agents.[40,41] By contrast, depletion of MAP4 has been associated with decreased polymeric tubulin.[42]

Regulation of MAP4 through p53 transcriptional status has been shown to determine sensitivity to antimicrotubule drugs.[40] When p53 is inactive, increased MAP4 expression increases microtubule polymerisation and facilitates paclitaxel binding, which increases sensitivity to paclitaxel and decreases sensitivity to vinca alkaloids. Therefore, the action of antimicrotubule drugs could be enhanced by controlling p53-dependent regulation of MAP4. Indeed, such regulation has been investigated in a preliminary trial in which doxorubicin was used to induce p53 to repress MAP4, which was then followed by sequential treatment with vinorelbine.[43] Partial response was observed in 7 out of 16 patients and the therapeutic regimen was well tolerated.

Another example of how the action of antimicrotubule drugs can be enhanced through the manipulation of microtubule-regulatory proteins involves stathmin, a major tubulin-regulatory protein that is highly overexpressed in leukaemia.[44,45] Overexpression of stathmin has been associated with decreased microtubule polymerisation and reduced paclitaxel sensitivity.[46] Downregulation of stathmin has been shown to sensitise K562 leukaemia cells to paclitaxel, resulting in a synergistic inhibition of growth and clonogenic potential.[47] This indicated that the efficacy of antimicrotubule drugs may be enhanced by manipulating the expression of microtubule proteins and that identifying differential expression of proteins may predict treatment response.[1]

6. Conclusions

The tubulin/microtubule system remains an important target for anticancer therapies, although acquired and intrinsic resistance to antimicrotubule agents still poses a significant clinical problem. Several strategies exist to overcome this challenge and maintain the clinical importance of cytotoxic therapy targeted at the microtubule. New antimicrotubule agents are being developed that inhibit the growth of tumours refractory to vinca alkaloids and taxanes.

In addition, improving the current understanding of how aberrant expression of specific β-tubulin isotypes affects the action of antimicrotubule drugs could potentially improve therapeutic approaches (e.g., investigating the downregulation of class III β-tubulin as a means of increasing sensitivity to microtubule-stabilising agents). Microtubule changes that affect stability may also be exploited to improve treatment response (e.g., cells expressing less-stable microtubules are hypersensitive to microtubule-destabilising agents). Other potential strategies for overcoming resistance include gene silencing or small molecule approaches that target-specific regulators of microtubule function (e.g., downregulating stathmin to increase the sensitivity of leukaemia to microtubule-stabilising drugs).

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