INTRODUCTION

Among the oldest of the world's writings mentioning both benign and malignant tumors are several Egyptian papyrus scrolls, dating from approximately 1600 BC. Hippocrates, an ancient Greek physician, the "father of modern medicine", first gave the name *karkinos* and *karkinoma* (the ancient Greek words for "crab") to a group of diseases that are now known as cancer. He thought the disease spread out from a tiny spot like a crab and eventually took over the whole body.

The hallmarks of malignant neoplastic tissue are unregulated cell proliferation, invasiveness and metastasis to distant sites in the body. To put it simply, cancer is inappropriate cellular proliferation. Once an organism reaches maturity and stops growing, the amount of cell proliferation in its body is restricted to a few specific populations in which continuous turnover is required. Most of the cells in the body remain in a quiescent, non-proliferating state, which corresponds to G_0 in the cell cycle. Examples of normally quiescent cell populations are neurons and muscle cells. Examples of non-quiescent cell populations are intestinal epithelial cells and dermal cells. Normally proliferating cell populations are subject to stringent growth control mechanisms. Cancer is an abnormal state in which uncontrolled proliferation of one or more cell populations interferes with normal biological functioning. The proliferative changes are usually accompanied by other changes in cellular properties, including reversion to a less differentiated, more developmentally primitive state. Although cancer is a generic term encompassing many different diseases, a unifying feature of many tumors is the uncontrolled proliferation of their cells. As they proliferate, cancer cells disrupt the normal function of surrounding tissues (or distant tissues in the case of metastases), leading to eventual organ failure and death. Based on the notion that some tumor cells may proliferate more rapidly than normal cells, a common strategy for cancer chemotherapy has been to develop drugs that interrupt the cell cycle. A particularly attractive cellular target is the microtubule network, the main component of the mitosis stage of the cell cycle, during which the mitotic spindle (a bipolar apparatus constructed of microtubules) separates the replicated chromosomes.

MICROTUBULES

Microtubules are major dynamic structural components of the cytoskeleton involved in a variety of cell functions important for the development and maintenance of cell shape, cell reproduction and division, cell signaling, intracellular transport and cell movement. The biological functions of microtubules are regulated for the most part by their polymerization dynamics. Microtubules are built by the self-association of individual α/β-tubulin dimers. Tubulin is one of the most abundant cellular proteins accounting for 2-5% of total cell protein in most epithelial cells, while tubulin accounts for approximately 20% of total cell protein in the brain. The central role of tubulin in the cell division cycle, together with the fact that aberrant cell division is the hallmark of cancer has made tubulin and microtubules prime targets for cancer chemotherapy. In fact, microtubule-targeting drugs are the most effective class of anticancer agents. Among the most successful microtubule-targeted drugs, taxanes are arguably the most effective anticancer agents introduced in the clinic since cisplatin, due to their remarkable activity in a broad range of cancer malignancies. The list of compounds that bind tubulin is large and continues to expand. The overwhelming majority of them are natural products and their chemical structures are remarkably diverse. Microtubule-targeting drugs act cytotoxically by either destabilizing or hyperstabilizing microtubules, resulting in mitotic arrest at the prometaphase or metaphase to anaphase transition, leading to subsequent apoptotic cell death.

Microtubule structure

Microtubules are highly dynamic cytokeletal fibers whose dynamic properties are based on their inherent structure and polarity. Microtubules are built by the self-association of α and β tubulin dimers that associate in a head to tail fashion to form protofilaments. Thirteen protofilaments (in most eukaryotic cells), interact with each other laterally by contacts between monomers of the same type to form a hollow helical cylindrical microtubule with an outer diameter of 25nm. The assembly of the α-β tubulin heterodimers creates a polarity on the microtubule that greatly influences the polymerization rates of the two ends of the microtubule. The faster growing end is

referred to the plus end and the slower growing end is referred to the minus end. The minus end is usually located near the microtubule organizing center near the nucleus, while the plus end is spread out through the cell. **(Figure 1)**

The αβ heterodimer is the basic structural unit of the microtubule. Each subunit has a binding site for one molecule of GTP. Once the αβ dimer is formed, the nucleotide in the alpha subunit (GTP) is buried at the intradimer interface, and it cannot be hydrolyzed. In contrast the nucleotide on the β-tubulin is partially exposed on the surface of the dimer and be hydrolyzed from GTP to GDP at the so-called exchangeable site (E-site). The energy released after the hydrolysis of the GTP at the beta-tubulin is used to polymerize the αβ dimers and assemble them into microtubules. After hydrolysis, the nucleotide at the β-tubulin E-site is buried at the interdimer interface and becomes nonexchangeable.

Microtubule dynamics: dynamic instability

The biological functions of microtubules in all cells are determined and regulated in large part by their polymerization dynamics. Microtubules are in a state of dynamic instability, in which individual microtubules are either growing or shrinking and stochastically switch between two states. (**Figure 2A)** The switch from growth to shrinkage is called a catastrophe, and the switch from shrinkage to growth is called a rescue. Dynamic instability is due to the structural differences between the growing and the shrinking ends. If the nucleotide hydrolysis proceeds more rapidly than the subunit addition, the GTP cap is lost and the microtubule beings to shrink. But GTP-subunits can still be added to the shrinking end, and if enough subunits are added to form a new cap, then the microtubule growth resumes. In an intact microtubule, protofilaments made from GDP are forced into a linear conformation by the many lateral bonds within the microtubule wall. At the end of the microtubule there is a stable GTP cap. Loss of this GTP-cap, however, allows the GDP-containing protofilaments to relax into their more curved conformation that leads to a progressive disruption of the microtubule.

Several parameters have been used to characterize the dynamics of microtubule assembly: growth rate, shortening rate, frequency of transition from growth to shortening (catastrophe frequency (1), frequency of transition from shortening to growth or an

attenuated (pause) state (rescue frequency (1)), and the duration of the attenuated state when neither microtubule growth nor shortening can be detected (2). Overall microtubule dynamics due to dynamic instability is best described as "dynamicity," which measures the sum of visually detectable tubulin dimer exchange per unit time at the ends of microtubules.

These dynamic properties are crucial for microtubules to carry out many of their cellular functions such as reorientation of the microtubule network when cells undergo migration or morphological changes and the dramatic microtubule rearrangement at the onset of mitosis (3). Mitotic microtubules are 10-100 times more dynamic than interphase microtubules; they exchange their tubulin with the soluble tubulin pool with half-times of ~15 s during mitosis as compared with 3 min to several hours in interphase (4-7). The rapid microtubule dynamics in mitosis is thought to be critical for both the morphogenesis and activities of the bipolar spindle, which directs the alignment of chromosomes at the metaphase plate and their final segregation into two daughter cells.

Microtubule dynamics: treadmilling

In addition to the dynamic instability, microtubules have another kind of dynamic behavior, called treadmilling, which is net growth at one microtubule end and balanced net shortening at the opposite end. (**Figure 2B).** In involves the intrinsic flow of tubulin subunits from the plus end of the microtubule to the minus end and is created by differences in the critical subunit concentrations at the opposite microtubule ends. This behavior occurs in cells as well as in vitro, and might be particularly important in mitosis, and in transport of organelles throughout the cell.

Figure 1. The structure of microtubule. *Microtubules are hollow cylindrical structures, built from two kinds of similar 50-kd tubulin subunits, α- and β-tubulin, which associate in a head-to-tail fashion to form a protofilament. The lateral association of protofilaments (usually 13) produces a microtubule with an outer diameter of 25 nm. Microtubules are polar structures with a dynamic plus end and a minus end that can be stabilized by embedding it in a microtubule-organizing center (MTOC).*

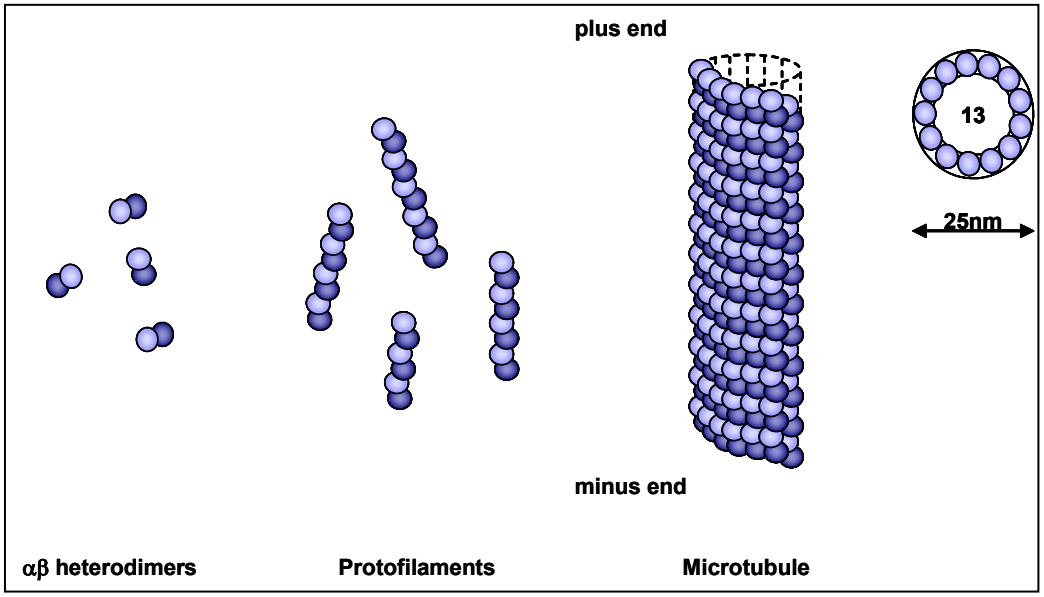
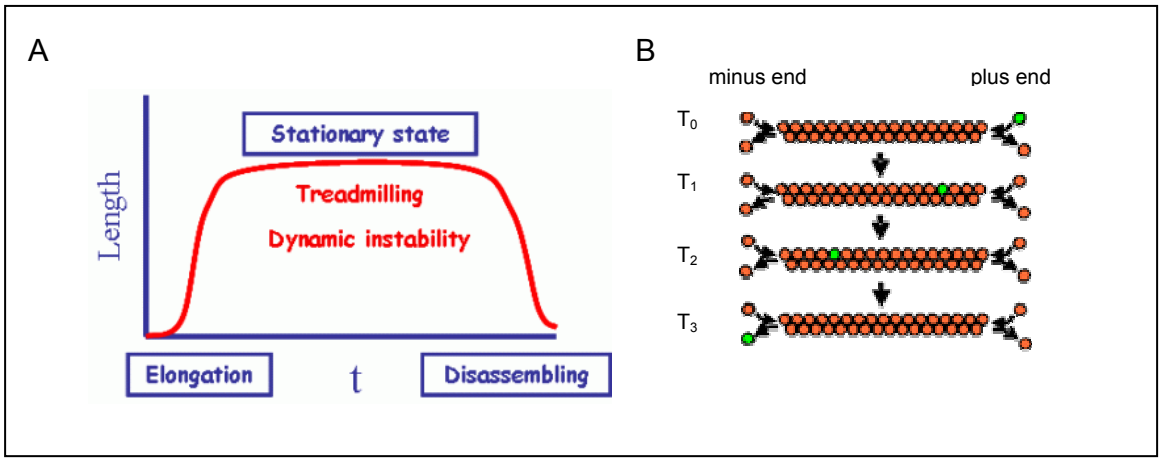


Figure 2. Dynamic Instability and Treadmilling in Microtubules. A) *Microtubules are in a state of dynamic instability, in which individual microtubules are either growing or shrinking and stochastically switch between two states. Microtubules grow and shorten by the reversible noncovalent addition and loss of tubulin dimers at their ends.* ***B)*** *Treadmilling at steady state in vitro showing a unidirectional flow (or flux) of tubulin subunits from plus to minus ends. Shown are consecutive ''snapshots'' of a microtubule exhibiting net growth at its plus end and equivalent net shortening at its minus end; the green subunits represent a marked segment and the microtubule remains at a constant length. T0 = zero time; T1, T2, and T3 =equal arbitrary increments of time.*



Microtubule Assembly

Assembly of microtubules in vivo occurs at the microtubule-organizing center (MTOC). In most animals cells there is a single, well-defined MTOC called the centrosome, located in the cytoplasm near the nucleus. The centrosome is a complex structure organized by a pair of perpendicular centrioles surrounded by pericentriolar material containing gamma tubulin in a large complex that includes other proteins (collectively known as grips) that form the gamma-tubulin ring complex (gamma-TuRC). The pericentriolar material contains several copies of a gamma-tubulin ring complex (gamma-TuRC), a multiprotein complex that nucleates microtubules and allows their elongation. In most cells, microtubules are organized into single array with their minus ends associated with the centrosome. Nucleation plays a fundamental role in the function and intracellular dynamics of microtubules by preventing spontaneous polymerization of microtubules in the cytoplasm and thereby a random spatial organization of microtubules. This gives a cell a defined polarity, with the minus end of the microtubules located near the nucleus in the center of the cell and their plus ends toward the cell periphery near the plasma membrane.

Isotypes of tubulin

Tubulin is encoded by a multigene family that produces a distinct set of gene products, or isotypes, of both α and β tubulin subunits. In mammals, there are at least six isotypes of α-tubulin and seven isotypes of β-tubulin. **(Table 1)** The reason for the existence of such a large number of tubulin isotypes is not very clear, although they are differentially expressed in cells and tissues and there is evidence that the tubulin isotypes play an important role during embryogenesis. However, the protofilaments of an individual microtubule can easily contain α and β tubulin subunits from different tubulin isotypes. The different isotypes of α and β tubulin are highly conserved and most of the sequence variation among them is fond in the last 10-15 carboxyl terminal (C-terminus) amino acids. The C-termini of tubulin are the primary binding location of the microtubule-associated proteins (MAPs). While the C-terminal regions are highly variable among the isotypes within a species, the same regions are highly conserved within a single isotype, among species are diverse as human, mouse and chicken. Variations among tubulin

isotypes are expected to affect primarily the association of accessory proteins on the surface of the microtubule rather than the microtubule polymerization per se. Some MAPs are more highly expressed in certain tissues and cells bind with higher affinity to certain tubulin isotypes, possibly explaining the array of tubulin isotypes that exist. (8)

In the case of cancer chemotherapy drugs, in vitro studies have shown that alteration of alpha and beta tubulin isotype composition has the potential to affect the sensitivity of tubulin to microtubule targeting drugs, especially taxol (9). Furthermore, it has also been proposed that altered expression of different tubulin isotypes represents another mechanism underlying the resistance of cancer cells to microtubule-targeting drugs. However, later studies in ovarian cancer xenograft models suggested that altered expression of β-tubulin isotypes does not influence taxol's sensitivity in vivo, arguing against a role for tubulin isotype composition in chemoresistance in the clinical setting (10)

	Class	Human gene	Expression
α-tubulin	1	TUBA1	Widely expressed
	1	TUBA3	Mainly in brain
	3	TUBA2	Testis-specific
	4	TUBA4	Brain; muscle
	6	TUBA6	Widely expressed
	8	TUBA8	Heart; muscle; testis
β-tubulin	I	HM40	Constitutive; predominant isotype in many cells
	II	Hβ9	Major isotype of neurons
	III	Hβ4	Neurons; Sertoli cells of testis
	IVa	Hβ5	Brain-specific
	IVb	Hβ2	Constitutive high levels in testis
	V	ND	ND
	VI	Hβ1	Hematopoiesis-specific cell types
	4Q	TUBB8	ND

Table 1. Tubulin Isoforms

In addition to the alpha and beta tubulin gene families, recent genetic analyses and database searches have added four new members of the tubulin superfamily, which now includes α, β, γ, δ, ε, ζ, η tubulin. Gamma and eta tubulin seem to be associated mainly with flagella and cilia motility (11), whereas sigma and epsilon tubulin were discovered by database searches, and their cellular functions have yet to be established (12-14). Gamma tubulin was first identified in the filamentous fungus Aspergillus idulans (15) and it is approximately 30% identical to alpha and beta tubulin, and likely to be present in all eukaryotes. Gamma tubulin is located in centrosomes where it plays an essential role in initiation of microtubule assembly.

Post-translational modification of tubulin

The α/β-tubulin heterodimer, the building block of microtubules, is subject to a large number of post-translational modifications. These modifications include: acetylation/deacetylation, tyrosination/detyrosination, polyglutamylation, polyglycylation, palmitoylation, and phosporylation (for review see (16)). All of these modifications, with the exception of acetylation occur at the highly charged end of alpha and beta tubulin, which is located on the outside of the microtubule, where they are well positioned to influence interaction with other proteins, All of the post-translational modification are ubiquitous in all organisms, and with the exception of detyrosination/tyrosination, all of these post-translational modifications that occur in higher eukaryotes are already present in the protest Giardia lambdia, considered the representative of the oldest eukaryotes. Among these post-translational modifications, only tubulin acetylation/deacetylation has been implicated in the regulation of microtubule stability (16). Most α-tubulins can be acetylated on a conserved lysine residue at position 40 in the N-terminus. Acetylated α-tubulin is most abundant in stable microtubules, yet has been found absent from dynamic microtubules such as the microtubules in neuronal growth cones and in the leading edges of fibroblasts (17-19). The distribution of acetylated α-tubulin is tightly regulated in cells. However, the enzymes catalyzing the acetylation and deacetylation of tubulin have remained unidentified until recently. HDAC6, a newly identified member of the histone deacetylase family, was recently identified as a tubulin deacetylase (20, 21). HDAC6 was found to co-localize with microtubules in cells and deacetylate microtubules both *in vitro* and *in vivo*. Furthermore, HDAC6 function appears to be specific to tubulin, as it has no effect on the global acetylation of histones H3 and H4 (20). Overexpression of HDAC-6 in mammalian cells leads to tubulin hypoacetylation. In contrast, genetic or pharmacologic inhibition of HDAC-6 function leads to hyperacetylation of tubulin, which is associated with increased microtubule stability (20, 21)

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Microtubule functions

Microtubules are essential components of the cytoskeleton and play a critical role in a variety of cellular processes including cell division, cell motility, intracellular trafficking, and cell shaping

Cell division

A defining characteristic of living organisms is their ability to proliferate and the very fundamental basis is the division of a single cell into two daughter cells with exact complements of the parental genetic material. **(Figure 3)** At the beginning of the early prophase of the cell cycle, centrosomes are already replicated, have split into two and begin to separate. Each centrosome now nucleates its own radial array of microtubules called an aster. By early prometaphase the two asters are completely separated and forming the bipolar mitotic spindle. Three different kinds of microtubules are present in the mitotic spindle. Astral microtubules, radiating in all directions from the centrosomes, are thought to contribute the force that separates the poles and orients and situates the spindle within the cell. Polar microtubules interact in an antiparallel fashion at the equator of the spindle and stabilize the bipolarity of the spindle. Kinetochore microtubules attach end-on to the kinetochore and connect chromosomes to spindle poles. The behavior of each class of microtubules is thought to be different because of the different protein complexes that are associated with their plus and minus ends.

When cells divide, the chromosomes must be delivered flawlessly to the daughter cells. The mechanical attachment of a chromosome to the spindle determines its delivery to the daughter cells. The spindle of a dividing cell has two poles, and each duplicated chromosome has two attachment sites, kinetochores (**Figure 4A**). Attachment of the kinetochores to opposite poles results in the delivery of one copy of each chromosome to each daughter cell. Kinetochore microtubules, link each kinetochore with a pole. The asymmetry of microtubules (22, 23) leads to force production toward that pole. As a result, each chromosome moves to the pole to which its kinetochore is attached. Tension is the source of order in this world of chance. Tension is absent in the improper attachment (**Figure 4B**) but present in the proper one. We do not know how tension stabilizes the attachment of chromosomes to the spindle. Tension could prevent change

by stabilizing either the microtubules themselves or the anchorage of microtubules at the kinetochore or at the pole. A microtubule motor protein could link each microtubule and the pole**.** Some candidates for polar motor-linkers have been identified, such as Eg5 the mitotic kinesin (24, 25). It has generally been believed that the attachment between kinetochores of chromosomes and the plus ends of microtubules is highly dynamic, in that tubulin subunits can assemble and disassemble at the kinetochore region (26-28). Physical tension is generated across kinetochore pairs following microtubule attachment to kinetochores. The amount of tension generated between kinetochore pairs is probably regulated by the combined action of microtubule dynamics and microtubule motors within the vicinity of kinetochores (29, 30).

Spindle assembly and function require localized regulation of microtubule dynamics and the activity of a variety of microtubule-based motor proteins. One of the consequences of entering cell division is that microtubules greatly increase their dynamic instability, driven primarily by an increase in the catastrophe rate. This increase in dynamic instability is regulated by different factors including Op18/Stathmin, XKCM1 (KinI). Counteracting the destabilizing effects of these two catastrophe factors are MAPs that bind and stabilize microtubules (31)

Intracellular transport

The inherent polarity of microtubules allows for the directional flow of information within the cell (32), and microtubule motors from the dynein and kinesin families use this polarity to transport cargoes to and from the nucleus **(Figure 5)**.

Dyneins

The dyneins are minus-end directed motors, while the kinesins are primarily plus-end directed motors. Both of these "taxis" use ATP-hydrolysis to generate force and move in a step-like manner on microtubules (33). Dynein drives a wide variety of cytoplasmic motor activities ranging from movement of ER-Golgi complexes, late endosomes and lip droplets (34, 35) to viral capsids (36) and chromosomes (37, 38). . To drive such a variety of subcellular motile functions, dynein works in concert with an accessory factor:

dynactin **(Figure 5B)**. Dynactin is thought to serve as an adapter that mediates dynein binding to a variety of cargo structures, including membranes, chromosomes and microtubules (reviewed by (39, 40)). It is also proposed to facilitate long-range movement by increasing dynein processivity (41). To allow for these different functions, dynactin has two distinct structural domains (42). Its rigid, filamentous backbone contains several proteins whose sequences predict both covalent and noncovalent cargo attachment mechanisms (37), whereas its flexible projecting sidearm binds dynein and microtubules (reviewed by (40)). These two domains are thought to be linked by the protein dynamitin which, when overexpressed, causes dynactin's sidearm to release from the backbone (43), thus decoupling dynein-binding and cargo-anchoring functions (44). This leads to a variety of defects; mitotic cells arrest in pseudoprometaphase (43), whereas interphase cells show altered steady-state distributions of the Golgi complex, endosomes, and lysosomes (45). In cells that overexpress dynamitin, bidirectional movement of late endosomes and of other organelles, is completely inhibited (35).

Kinesins

On the other hand kinesins, constitute a large family of motor proteins that move cargoes towards the microtubule plus end, located in the cell periphery in most epithelial cell types **(Figure 5A)**. Examples of cargoes that move within the cell via kinesins are the mitochondria and the tumor suppressor protein APC (46).

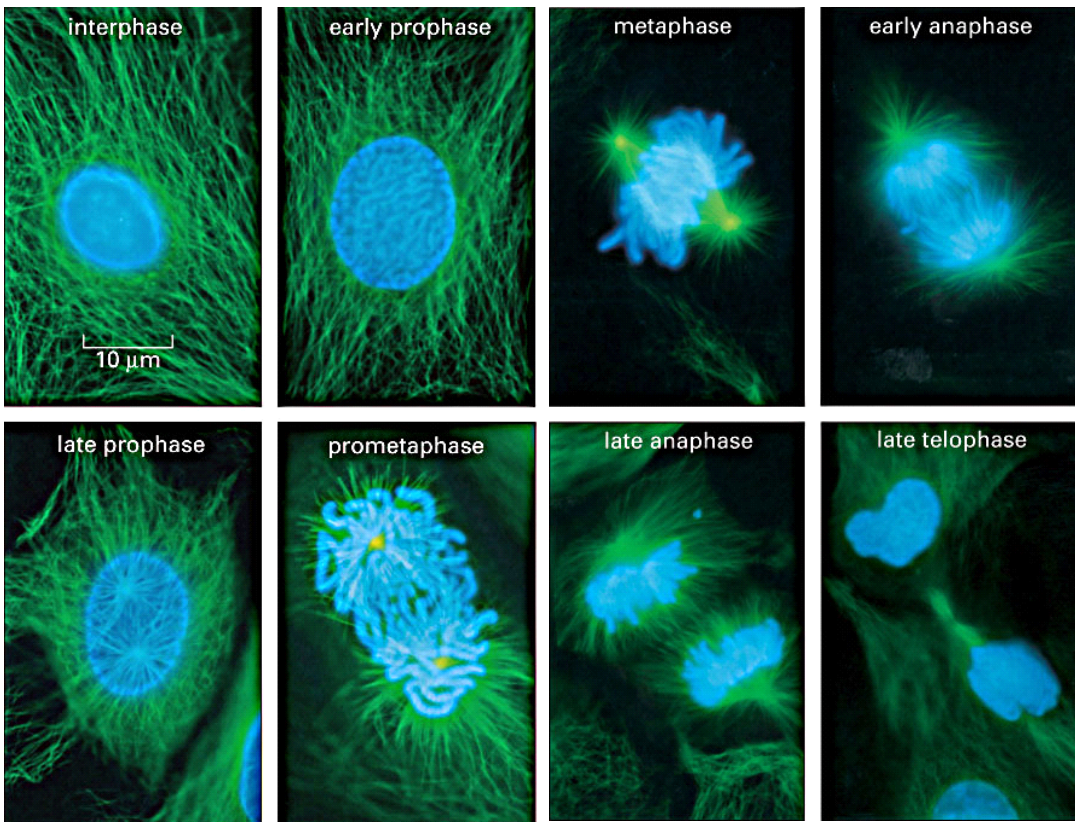
The collective transport of intracellular particles along a specific cellular direction is a fundamental process in cell biology. Interphase microtubules are the major component of the cytoskeletal system that is responsible foe the regulation and distribution of mitochondria in mammalian cells (47), and discovery of KifIB and Kif5B proteins belonging to the kinesin protein family, which are responsible for the movement of mitochondria along microtubules (48, 49) has accelerated the research on factors controlling the distribution of intracellular organelles besides mitochondria: endoplasmic reticulum (ER) (50), Golgi apparatus (51), perozisomes (52) and lysosomes (53).

Overexpression of tau, a microtubule-associated protein, not only causes aberrant distribution of mitochondria via inhibition of kinesin-dependent movement of the organelles along the microtubules, but also affects the morphology and distribution of ER

in the cell (54). Chemical modification of microtubule dynamics by microtubule-targeting drugs have been widely used as an experimental approach to explore the role of microtubule in various cellular functions (52, 54-56)

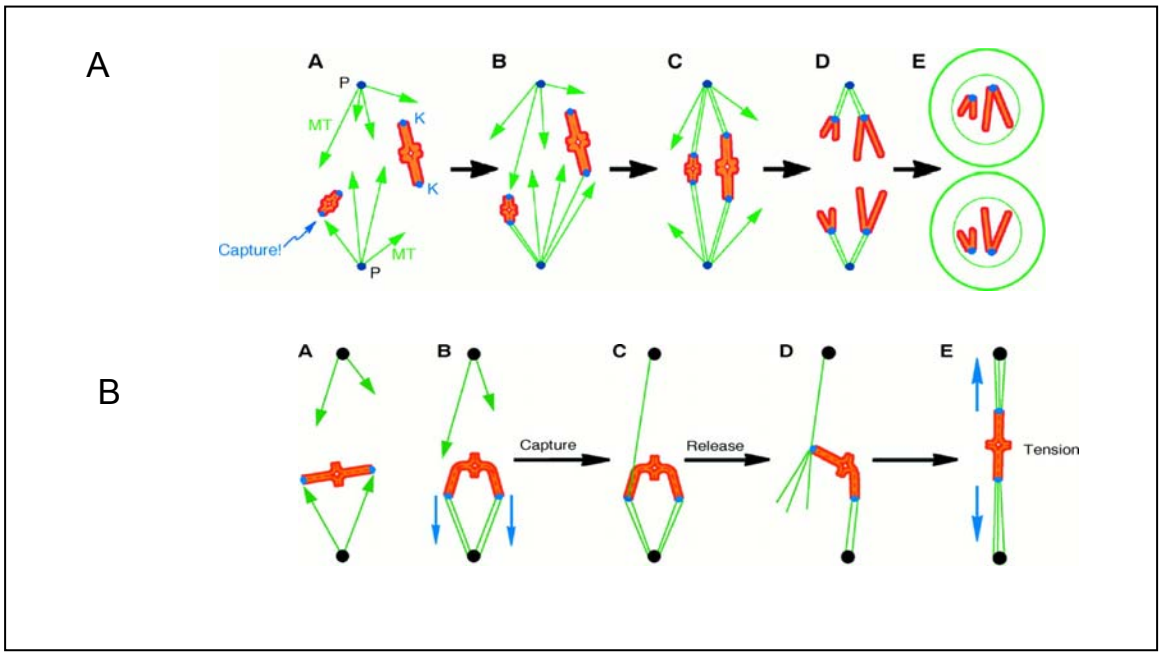
In addition to that, microtubules have been shown to be essential for the localization and transport of a great variety of proteins and transcription factor within the cells. The list of these proteins is growing very rapidly and includes p53 (57), Hsp90 (58, 59), pVHL (60), HIF1-α (61). In a similar way, microtubules are also used by intracellular bacteria and viruses, and are important to the life cycle of these pathogens (62).

Figure 3. Phases of Mitosis *In these micrographs microtubules (green) and DNA (blue) have been visualized by immunofluorescence. During the interphase, the centrosome organized the interphase microtubule array. By early prophase, the single centrosome contains two centriole pairs. At late prophase, the centrosome divides and the resulting two asters can be seen to have moved apart. At prometaphase, the nuclear envelope breaks down, allowing the spindle microtubules to interact with the fully condensed chromosomes. At metaphase, the bipolar structure of the spindle is clear and all the chromosomes are aligned at the equator of the spindle. At early anaphase, the sister chromatids all separate synchronously and, under the influence of the microtubules, the daughter chromosomes begin to move towards the poles. By late anaphase, the spindles poles have moved farther apart, increasing the separation of the two groups of chromosomes. At telophase, the daughter nuclei re-form and by late telophase, cytokinesis is almost complete, with the midbody persisting between the daughter cells.*



Moleculat Biology of the Cell. Figure 18-8. 4th Edition

Figure 4. Chance, attachment, and chromosome transmission. ***A)*** *The normal, errorless course of events is illustrated for a cell in mitosis.* ***(a)*** *The basic elements are a spindle with two poles (P) and paired chromosomes, each with two spindle attachment sites, kinetochores (K). Microtubules (MT) growing from a pole may by chance encounter a kinetochore and be captured, thereby attaching the chromosome to that pole.* ***(b to d)*** *If all goes well, the two kinetochores on each chromosome pair become attached to opposite spindle poles; in anaphase, each chromosome pair splits into partner chromosomes that move to opposite poles.* ***(e)*** *Each daughter cell has a copy of each chromosome.* ***B)*** *By chance, a chromosome's kinetochores may capture microtubules from the same spindle pole. Left uncorrected, this would result in one daughter cell with two copies of that chromosome and one daughter with none.* ***(b to d)*** *Tension is absent in such defective attachments; the forces (blue arrows)* ***b)*** *are directed to the same pole. The result of no tension is instability, characterized by repeated bouts of capture and release until the proper attachment is reached, when tension puts an end to change. Error correction requires two chance events, the capture of new microtubules and release from the old attachment.* ***(e)*** *When the proper attachment arises, it is stabilized by tension from opposed mitotic forces (blue arrows).*



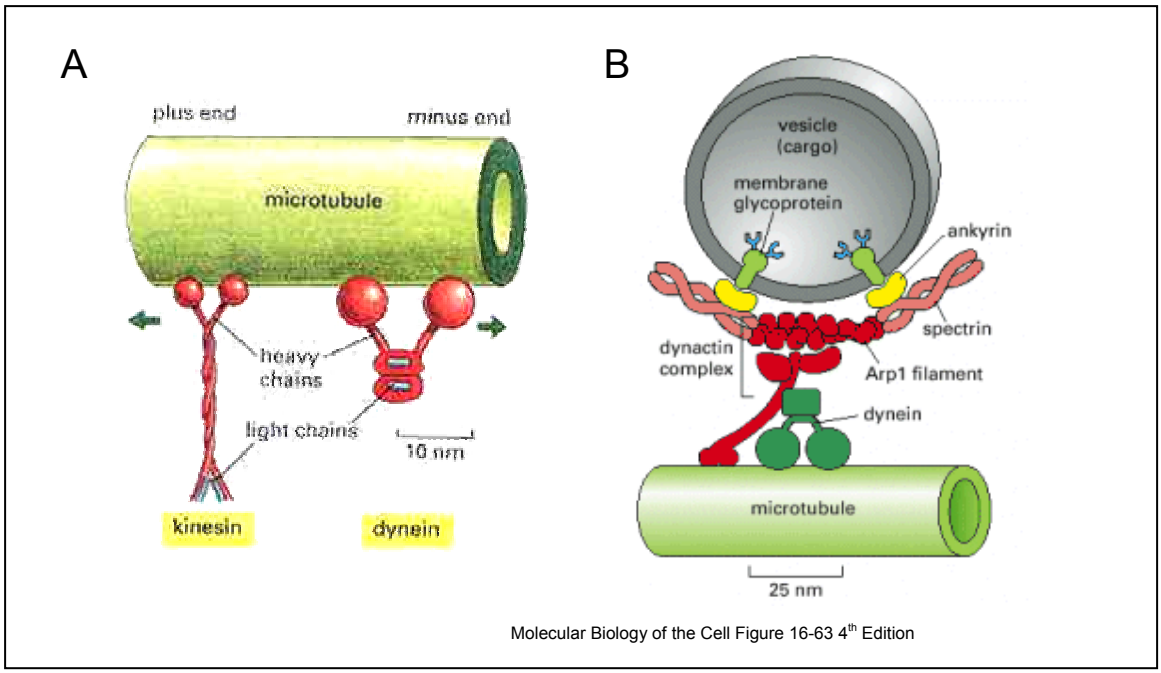


Figure 5 Kinesins and Dyneins are Microtubule Motors. ***A)*** *The kinesins are motor proteins that move towards the plus end of microtubules. Kinesin has two heavy chains and two light chains per active motor, two globular head motor domains, and an elongated coiled-coil responsible for heavy chain dimerization. Most kinesins carry a binding site in the tail for either a membrane-enclosed organelle or another microtubule. Many of the kinesin superfamily members have specific roles in mitotic and meiotic spindle formation and chromosome separation during cell division. The dyneins are a family of minus-end-directed microtubule motors. They are composed of two or three heavy chains (that include the motor domain) and a large and variable number of associated light chains.* ***B)*** *Dynein requires the presence of a large number of accessory proteins to associate with membrane-enclosed organelles. Dynactin is a large complex (red) that includes components that bind weakly to microtubules, components that bind to dynein itself, and components that form a small actin-like filament made of the actin-related protein Arp1.*

RNA COMPLEXES AND THE CYTOSKELETON

Role of the Microtubule Cytoskeleton in the Control of Protein Translation

The functional importance of the cytoskeleton and its associated proteins is becoming better understood over the years. Recently discovered roles of the actin and microtubule networks are transport and localization/anchoring of RNA. Approximately 15-30% of cellular mRNAs and polysomes are thought to be associated with the cytoskeleton, and immunohistological and biochemical approaches suggest that translation initiation and elongation factors in certain cell types follow a microtubule pattern (63). Proteins that regulate translation and also associate with microtubules include elongation initiation factors, such as eif-2, eif3, eif4a, eif-5, and cap-binding proteins eif4e, eef1α, and eef1-2 [for review see (63)]. Perhaps most of the understanding about cytoskeletal-mRNA interactions comes from studies where compartmentalization of specific RNAs involved in proper embryonic development, such as bicoid mRNA and oskar mRNA, require intact microtubules capable of trafficking ribonucleoprotein granules to distinct locales within the cell (64, 65). Furthermore, some mRNA species such as c-myc, cyclin A, histone H4, and ribosomal protein S6 mRNAs are believed to predominantly associate with cytoskeleton-bound polysomes as opposed to membrane-bound or free polysomes (66, 67).

The association between mRNAs and the cytoskeleton is thought to occur via specific sequences or secondary structures in the mRNA 3'UTR (63). For all of the mRNAs targeted to actin and/or microtubules known to date, deletions in only the 3'UTR and not in other parts of the mRNA result in incorrect localization (63, 68). Although it is possible that sequences in the RNA itself facilitate direct binding to microtubules, recent studies suggest that RNA binding proteins act as linkers between the mRNA and the cytoskeleton. Examples of known RNA binding proteins that provide this association include Staufen, TB-RBP, ZBP-1, CPEB, and hnRNP A2 (63, 69, 70). For HIF-1α, under normoxia, the majority of translation occurs by assembly of the ribosome at the 5' mRNA cap (cap-dependent translation). **(Figure 6)** It has been demonstrated recently that prolonged hypoxia specifically inhibits cap-dependent translation through changes in the assembly of the eIF4F 5' mRNA cap binding complex. In addition, HIF-1α translation seems to be spared the general reduction in translation rate that occurs during hypoxia due to an internal ribosome entry site (IRES) on its mRNA (Lang et al., 2002). HIF-1α is therefore translated

and stabilized during hypoxia and can dimerize with HIF-1β to induce transcription of HRE-responsive genes.

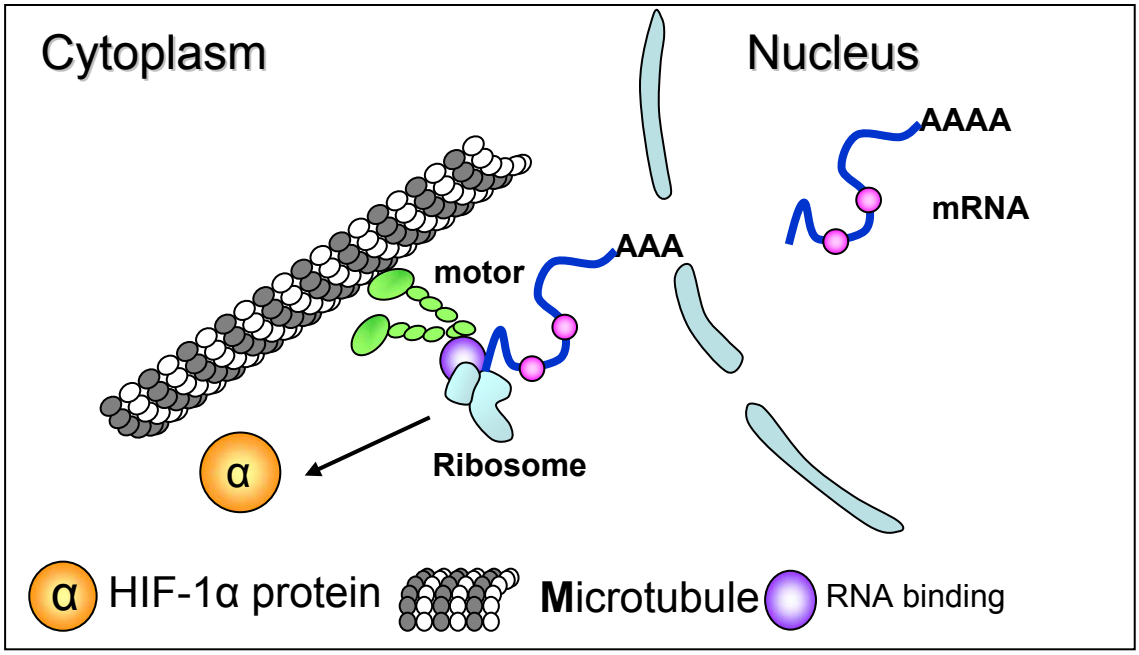


Figure 6. Microtubule-Dependent HIF-1α Translation. *Several mRNAs associate with microtubules. For example the myelin basic protein (MBP) mRNA contains kinesins granules for translocation. The granules enhance translation efficiency by spatially clustering certain mRNA species together with translational machinery; they also facilitate travel to distinct regions of the cell via microtubule motor proteins. We have found that HIF-1a mRNA also associates with microtubules via an unknown kinesin motor*

TARGETING MICROTUBULES FOR CANCER CHEMOTHERAPY

Microtubules are crucial for a variety of functions in the cell, including the maintenance of cell shape and polarity, intracellular transport of vesicles and organelles, and signal transduction. However, microtubules are especially important during cell division, which requires an exquisite control of microtubule dynamics. During cell division microtubules form the bipolar mitotic spindle, the structure that is required to faithfully segregate sister chromatids into the two daughter cells. The critical role that microtubules play in cell division makes them a very suitable target for the development of chemotherapeutic drugs against the rapidly dividing cancer cells. Microtubule-targeting drugs are arguably the most effective class of drugs in cancer therapy. For no other single target, including DNA, has a more diverse group of agents successfully been developed. The effectiveness of microtubule-targeting drugs has been validated by the successful use of several taxanes and vinca alkaloids for the treatment of a wide variety of human cancer.

The overwhelming majority of microtubule-targeting drugs are natural products and their chemical structures are remarkably diverse. These tubulin-targeting agents are divided into two groups: those that bind to the tubulin dimers and destabilize microtubules such as the Vinca alkaloids, cryptophycins, and colchicine and those that bind to the microtubule polymer and stabilize microtubules, such as the taxanes (Taxol and Taxotere), epothilones, eleutherobins, laulimalide and discodermolide. These drugs bind to different sites on the tubulin dimer and at different positions within the microtubule, and they have diverse effects on microtubule dynamics. However, they all block mitosis at the metaphase/anaphase transition and induce cell death (for review see (71). Clinical drug development saw the introduction of the Vinca alkaloids (vinblastine, vincristine, vinorelbine) in the 1950s. Although the Vinca alkaloids were shown to be useful in a wide range of malignancies, including both leukemias and solid tumors, interest in developing new agents targeting microtubules did not take off until the introduction of Taxol into clinical oncology (72). Originally isolated in 1971 from the bark of the Pacific yew tree, Taxus brevifolia (73), Taxol was the first natural product described that stabilized microtubules. This unique mechanism of action of Taxol as a microtubule-stabilizer was first reported in 1979 (74) and generated intense interest for its clinical

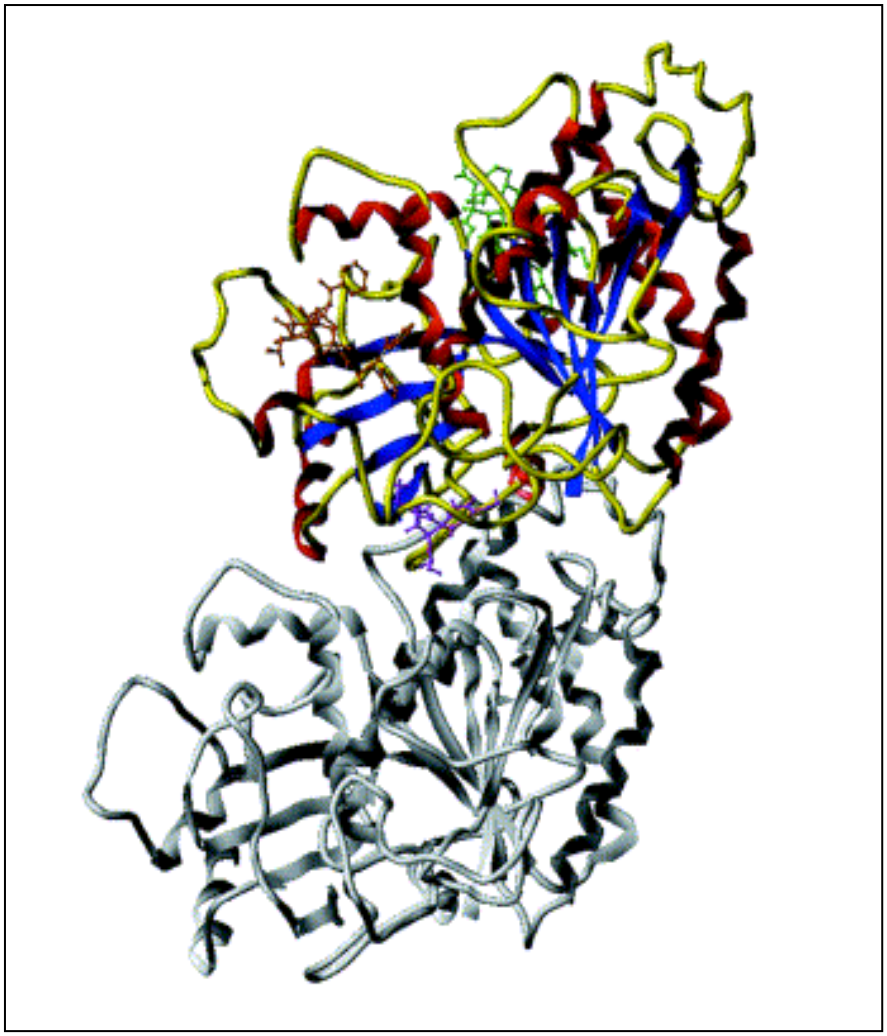
development.

Mechanism of action of microtubule-targeting drugs

The main mechanism of action of microtubule-targeting compounds is to block cell in mitosis at the G2/M phase of the cell cycle, for this reason these compounds are often referred to as anti-mitotic. Currently, there are five known drug binding sites on tubulin: four that are well-characterized and a fifth that is not yet thoroughly characterized. They have names assigned depending on which drug was originally found to bind the site. (**Figure 7**) The **taxane** binding site on β-tubulin is shared by drugs that stabilize microtubules and bind preferentially at the microtubule polymer (75-79). **(Figure 8).** The prototype of this class of drugs is Taxol (74), but newer members include the epothilones (80) discodermolide (81), eleutherobin, and the sarcodictyins (82)

All of the other well-characterized binding sites, with the exception of the fifth binding site, are shared by drugs that bind preferentially to unpolymerized tubulin, inhibiting tubulin assembly. These destabilizing agents either form covalent crosslinks to tubulin cysteine residues such as Cys-β239 (the small molecules 2,4-dichlorobenzyl thiocyanate (83) and T138067 (84)); bind tubulin at the **colchicine** site (**Figure 9**) (the combretastatins (85), curacins (86), 2-methoxyestradiol (87), and the podophylotoxins (88)); bind tubulin at the ***Vinca*** domain (**Figure 10**), (maytansin, rhizoxin (89, 90)); or locate in alpha-tubulin as do the hemiasterlins (91), which also bind at the Vinca domain (92), and perhaps the cryptophycins (for review see (93). In the case of drugs that bind at the *Vinca* alkaloid or the colchicine sites, it should also be noted that although they show a preference for unpolymerized tubulin, they can also bind microtubules to a lesser extent (71).

Figure 7. Microtubule drug-binding sites*. A hypothetical model of three drugs bound to the tubulin dimer. The relative positions of the three major classes of microtubule-interacting drugs are shown here: vinblastine (green), paclitaxel (orange) and colchicine (magenta). Paclitaxel binds at the lateral interface between adjacent protofilaments from the terminal end of the microtubule. Colchicine binds at the intra-dimer interface between β- and α-tubulin (grey). Vinblastine binds at the polar, opposite side of β-tubulin at the plus end interface, adjacent to the hydrolysable nucleotide site.*



The fifth binding site, on tubulin, has been recently identified as the location where the microtubule-stabilizing drug laulimalide binds (94). This is the first report of the existence of a second drug-binding site other than the taxane site on the microtubule polymer that promotes microtubule stability. Recently, a microtubule-stabilizing natural product derived from a New Zealand marine sponge, peloruside A, was also found to compete with laulimalide for this site (95).

Agents that bind to the colchicine site

Drugs binding to the colchicine site typically induce microtubule depolymerization at high concentrations, while they suppress microtubule dynamics at low concentrations. Isolated from the meadow saffron Colchicum autumnale, colchicine is one of the earliest microtubule-targeting agents identified. In fact tubulin was first purified based on its high affinity binding to colchicine and was referred to as "a novel protein binding colchicine" (96, 97).

Colchicine has been used to treat gout, however it has not been clinical developed for cancer treatment due to its high toxicity in normal tissues. On the other hand, the development of agents binding to the colchicine site as potential cancer chemotherapeutic drugs has recently gained intense interest. This is the case of combretastatins, isolated from the South African willow Combretum caffrum, that bind to tubulin and exhibit potent anti-tumor and antiangiogenic activity (98-100). Similarly, 2-methoxyestradiol-2 (2Me2), a naturally occurring metabolite of estradiol, also inhibits tumor growth and angiogenesis (61, 101).

Agents that bind to the Vinca alkaloid site

The vinca alkaloids, vinblastine and vincristine, were isolated over 40 years ago from the leaves of the periwinkle Catharanthus roseus. Since then they have been widely used to clinically treat leukemias, lymphomas and a few solid malignancies. The clinical success of these two natural products together with the elucidation of binding to microtubules has

facilitated the development of several semi-synthetic derivatives, notably vindesine, vinorelbine and vinflubine, which are now used in the clinic for the treatment of cancer (102). All the members of the vinca alkaloid family cause microtubule depolymerization, dissolve spindle microtubules and arrest cells in mitosis, In contrast, at low concentrations, the vinca alkaloids suppress microtubule dynamics without depolymerizing microtubules.

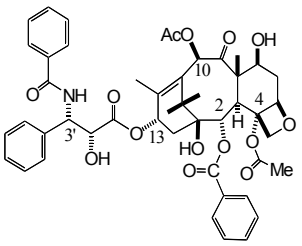
Agents that bind to the taxane site

Originally isolated in the 1971 from the bark of the Pacific yew Taxus brevifolia, paclitaxel did not receive much attention until it was discovered to possess microtubule-stabilizing activity. (73, 74) This drug is now in widespread use for the treatment of breast, ovarian, prostate, and non-small cell lung cancer, as well as Kaposi's sarcoma. Its semi-synthetic analog, docetaxel, is synthesized from a precursor isolated from the needles of the European yew Taxus baccata. Docetaxel is more water soluble than paclitaxel, and is also slightly more active than paclitaxel against cancer cell proliferation, and is now widely used in the clinic.

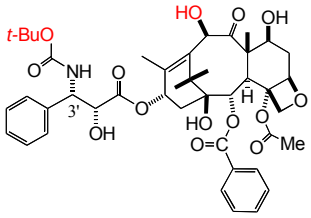
At relatively high concentrations, the taxanes promote microtubule polymerization and microtubule bundles in cells, although the biological significance of this phenomenon remains unclear. At lower concentrations, the taxanes suppress microtubule dynamics without affecting microtubule polymer mass.

The success of paclitaxel and docetaxel in cancer therapy has inspired the discovery of new microtubule-targeting agents with similar mechanisms of action, including epothilones (isolated from the myxobacteirum Sorangium cellulosum), discordermolide (isolated from the marine sponge Discodermia dissolute), eleutherobin (isolated from the marine soft coral Eleutherobia sp.), sarcodyctins (isolated from the Mediterranean stoloniferan coral Sarcodictyon roseum), laulimalide (isolated from the marine sponge Cacospongia mycofijiensis) and peloruside (isolated from the marine sponge Mycale sp.) With the exception of laulimalide and peloruside, all these compounds have been reported to bind at the taxane site (103). These taxane-like agents block mitosis and induce apoptotic cell death downstream of their binding to tubulin, and their cancer chemotherapeutic potential is currently under clinical investigation.

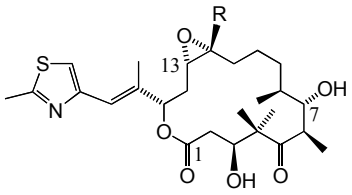
Figure 8 Microtubule polymerizing drugs. *All polymerizing drugs bind at the taxane-binding site of beta-tubulin, except for laulimalide, whose binding site is unknown.*



Taxol

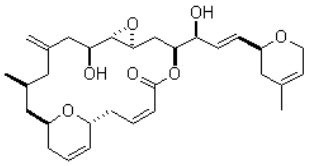


Taxotere

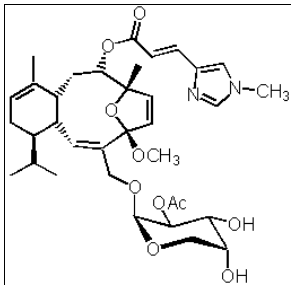


Epothilones

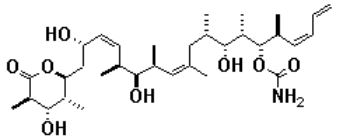
A R= H, **B** R = Me



Laulimalide

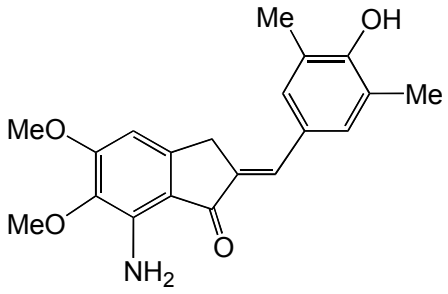


Eleutherobin

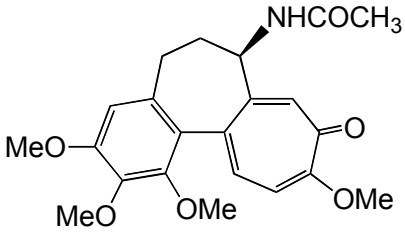


Discodermolide

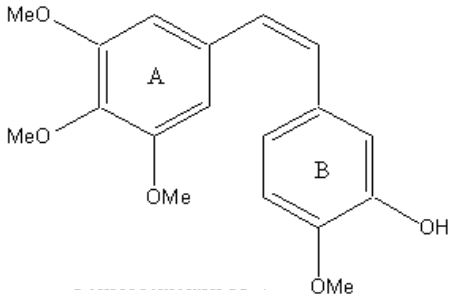
Figure 9. Microtubule depolymerizing drugs that bind at the Colchicine binding site on β-tubulin.



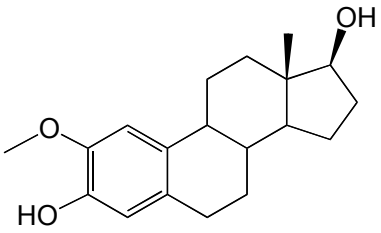
Indanocine



Colchicine

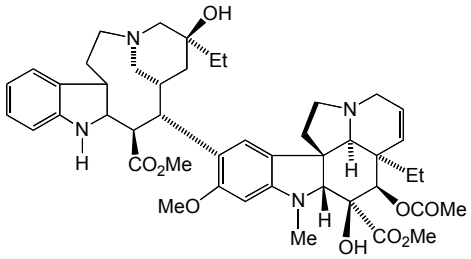


Combretastatin

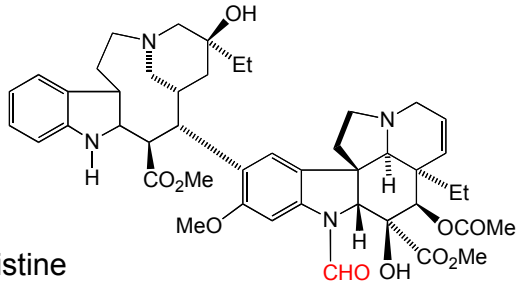


2-methoxy-estradiol

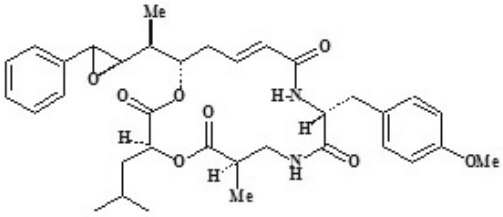
Figure 10. Microtubule depolymerizing drugs that bind at the Vinca alkaloid binding site on β-tubulin.



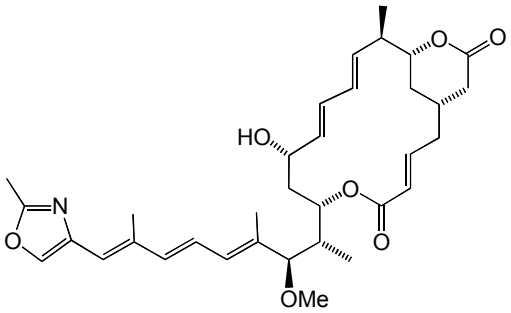
Vinblastine



Vincristine



Cryptophycin B



Rhizoxin

Clinical Applications of Microtubule-Targeting Drugs

The use in medicine of natural products containing microtubule-targeting drugs dates back to antiquity. In more recent years, the microtubule-destabilizing compounds have been widely used in the treatment of human disease, even before the protein tubulin was discovered as their target. Today, microtubule-destabilizing drugs are being used as antifungal and antihelminthic agents (104), while colchicine is also being used for treatment of both familial Mediterranean fever (105) and gout (106). However, the main use of microtubule-targeting drugs is in clinical oncology.

The *Vinca* alkaloids vinblastine and vincristine, the first antitubulin agents released for clinical trials in 1961, were approved by 1966 for the treatment of childhood acute leukemia. However, the mechanism of action of these *Vinca* alkaloids was not elucidated until later. Although the *Vinca* alkaloids had been used successfully for the treatment of both hematologic malignancies and solid tumors, interest in the development of new agents that target microtubules declined until the introduction of Taxol into clinical oncology.

Taxol's unique mechanism of action, first reported in 1979 (74) generated intense interest in its clinical development. In the ensuing years, Taxol was moved into the clinic, where its effectiveness has been continually improved. So far, the taxanes Taxol and Taxotere have been approved by the FDA for the treatment of ovarian, breast, prostate, head & neck, and non-small-cell lung cancer. We expect that these clinical indications will continue to expand (72, 107)**.**

Anticancer drug resistance is best defined as a state of insensitivity or decreased sensitivity of cancer cells to drugs that would ordinarily cause cell death. This resistance can be either intrinsic or acquired. Intrinsic resistance is defined as a state of insensitivity to initial therapy in response to a drug or combination of drugs. On the other hand, acquired drug resistance is defined as a state whereby a population of cancer cells that were initially sensitive to a drug undergoes a change towards insensitivity. Acquired drug resistance is the most common reason for the failure of drug treatment in cancer patients

with initially sensitive tumors, and as such, is presently responsible for the majority of deaths from cancer. The gravity of the drug resistance problem in clinical oncology has led to intense scientific efforts to understand the molecular mechanisms that lead to drug resistance, as well as to identify the ways to overcome it.

Drug-resistance to microtubule-targeting agents

The clinical success of Taxol is dampened by the emergence of drug resistant tumor cells. With the exception of P-glycoprotein (Pgp)-mediated multi-drug resistance (MDR) (108, 109) resulting in decreased intracellular drug accumulation, all other mechanisms appear to involve alterations in tubulin. Such alterations include: (1) differential expression levels of β-tubulin isotypes in Taxol-resistant cells (110-112); (2) an increase in microtubule dynamics in Taxol-resistant cancer cells (113), as well as decreased ability of Taxol to suppress microtubule dynamics in cells overexpressing βIII-tubulin (114); and most importantly, (3) the presence of tubulin mutations (115-119).

Research on tubulin mutations is not new. The tubulin protein, first isolated in 1968 as "the colchicine-binding protein" (96) has since been the subject of constant discoveries regarding not only its wide array of cellular functions, but also the variety of effects that different microtubule-targeting drugs have on it. The original discoveries of relationships between tubulin mutations and drug resistance actually began in the late 1970s. In fact, the first study reporting that tubulin mutations can confer resistance to a microtubule-targeting drug, benomyl, found this resistance to be due to a decrease in binding affinity in cells from the fungal strain *Aspergillus nidulans* (120). Following this initial discovery, two later studies described a reduction in the binding affinity of radiolabeled colcemid to tubulin (121), as well as an altered electrophoretic mobility of β-tubulin obtained from Chinese hamster ovary cells resistant to either colcemid or colchicine, respectively (122).

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In the ensuing years, discoveries of drug resistance to antimitotic agents due to alterations or mutations in tubulin continued to amass in studies involving lower organisms such as the yeast *Schizosaccharomyces pombe* (123) and the myxoamoebae *Physarum* regarding

resistance to benzimidazol (124), as well as in organisms resistant to benomyl such as *Neurospora crassa* (125) and *Saccharomyces cerevisiae* (126). However, it was not until 1997 that acquired tubulin mutations conferring Taxol resistance in human cancer cells were described for the first time (115).

Since then, several other groups have described acquired tubulin mutations in human cancer cell lines which confer resistance to either microtubule-stabilizing or microtubule-destabilizing drugs, making tubulin mutations one of the most frequently encountered mechanisms of antitubulin drug resistance *in vitro*. In the end, what this means is that no matter where an organism is on the phylogenetic tree, it may be capable of acquiring mutations in its tubulin genes as a mechanism that allows these organisms to survive in otherwise toxic circumstances, such as in the presence of the powerful tubulin-targeting drugs. Thus, the acquisition of mutations that alter cellular microtubules' response to drug binding appears to be a simple but universal mechanism for an organism's survival in the presence of these drugs.

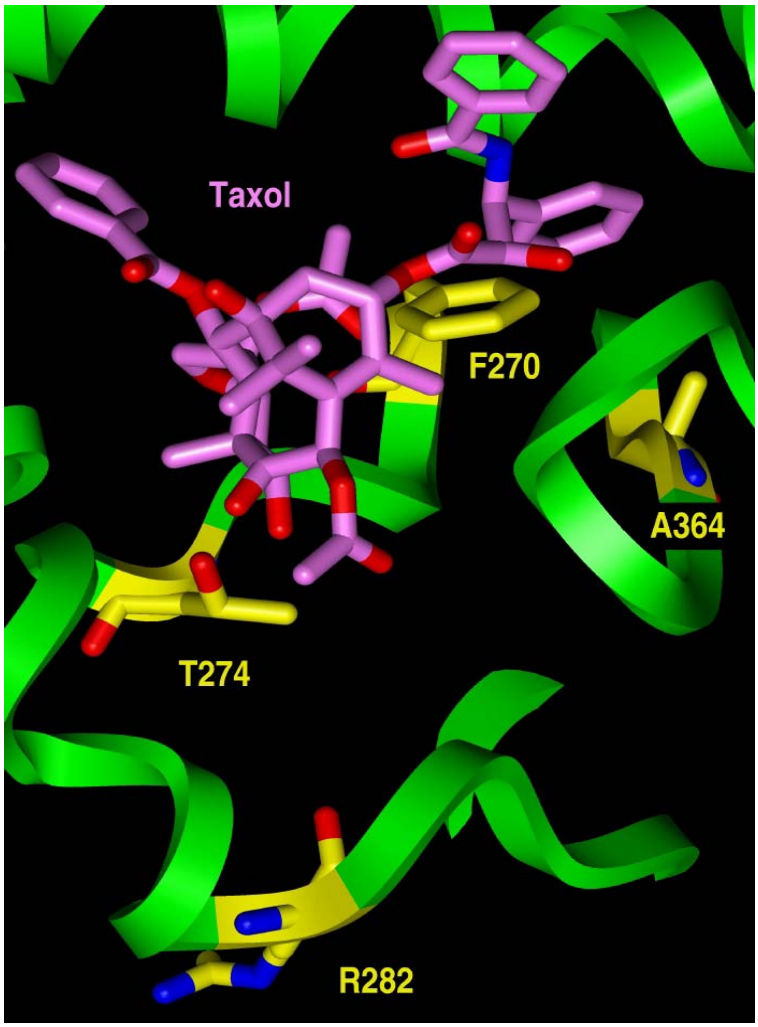
Mutations in Response to Selection with Taxol

The taxanes, **Taxol** and its semisynthetic analog **Taxotere (Figure 8)**, were among the most important new additions to the cancer chemotherapeutic arsenal after 1990. The taxanes bind preferentially and with high affinity to the β-subunit of the tubulin dimer, along the entire length of the microtubule. Electron crystallographic studies on Taxol complexed with tubulin have allowed the precise identification of the amino acids which comprise the taxane binding pocket on β-tubulin (79) and revealed the location of the site at the inside lumen of the microtubule (127). At high drug concentrations there is one molecule of Taxol bound on every molecule of tubulin dimer in a microtubule, which results in increased tubulin polymerization both *in vitro* and in cells at approximately a 1:1 stoichiometry. However, even at lower concentrations of Taxol where one molecule of Taxol is bound to every few hundred tubulin molecules along the microtubule, microtubule function can still be affected by a reduction in microtubule dynamics which ultimately leads to microtubule stabilization, mitotic arrest, and cell death (for review see (128).

Research on the mechanisms involved in taxane resistance has revealed that sequence alterations of single amino acids at the taxane binding site can have a significant impact on the drug's ability to bind tubulin. Two distinct β-tubulin mutations in two human ovarian cancer clones, namely clones 1A9/PTX10 and 1A9/PTX22, were selected independently with Taxol from the parental 1A9 human ovarian cancer cells. Sequence analysis of the predominantly expressed β-tubulin isotype (class I/gene HM40) revealed that both mutations were single nucleotide substitutions at residues **βPhe270Val** in 1A9/PTX10 cells, and **βAla364Thr** in 1A9/PTX22 cells (115) (see **Table 2**). As a result, impaired Taxol-induced tubulin polymerization was observed in the two clones, which were found to exhibit a 30-fold resistance to Taxol. To exclude other potential cellular alterations that could contribute to the observed Taxol resistance the mutant tubulins from each clone was purified and used to perform *in vitro* tubulin polymerization experiments with Taxol. The purified tubulins from clones 1A9/PTX10 and 1A9/PTX22 failed to polymerize *in vitro* in the presence of Taxol, in contrast to the 1A9 wild type (wt) parental cell tubulin, which was readily polymerized by Taxol under the same conditions. These results provided direct evidence that these specific acquired mutations were indeed responsible for the Taxol-resistance, due to a compromised drug binding site on tubulin. However, these two β-tubulin mutant clones exhibited very low cross-resistance to epothilone B (about 2-3 fold), even though epothilone competes with Taxol for the same binding site on β-tubulin. Consistent with this finding, epothilone B was still able to induce polymerization of these two isolated mutant tubulins *in vitro*. Thus, it appears that although the taxane binding site is the same for the two drugs, the specific amino acids necessary for binding of each drug to this pocket are distinct as determined by a comparison of epothilone A and Taxol bound to beta-tubulin (129) (**Figure 11**). Other acquired β-tubulin mutations in human breast cancer MDA-MB-231 cells (118) and human epidermoid cancer KB-3-1 cells (117), following Taxol selections have been described (**Table 2**). In the first case, a mutation was described in the class I β-tubulin gene at residue **βGlu198Gly** located near the α/β interphase (118). These β-tubulin mutant MDA-MB-231 breast cancer cells displayed 17-fold resistance to Taxol, some cross resistance to Taxotere and the epothilones and did not express Pgp. Introduction of wild-type class I β-tubulin into these resistant cells partially reversed the resistant phenotype, suggesting that this mutant residue was indeed responsible for the taxane resistant phenotype seen in these

cells. In a second study, Hari et al. (117) reported the identification of a novel point mutation **βAsp26Glu** in class I β-tubulin. This mutation is at the N-terminus of β-tubulin, which forms part of the taxane binding pocket (79), and confers an 18-fold resistance to Taxol with minimal cross-resistance to epothilone B and MAC-231, yet 10-fold cross resistance to Taxotere. Interestingly, these β-tubulin mutant KB3-1 cells are also drug dependent, as they require the presence of low concentrations of Taxol in the tissue culture medium for optimal growth. This finding would be consistent with the presence of less stable microtubules as has been reported in other studies of drug-resistant cell lines displaying a combination of Taxol-resistant and Taxol-dependent phenotypes (113, 130). Along the same lines, two related studies have described the characteristics of another Taxol selected human cancer cell line, the non-small cell lung cancer (NSCLC) A549 cells (113, 130). In these studies two clones derived from the A549 parental cells were isolated and characterized, namely A549-T12 and A549-T24. The A549-T12 cells were found to be 9-fold resistant to Taxol and did not express P-gp, while the A549-T24 cells were 17-fold resistant to Taxol and expressed low levels of P-gp. Sequence analysis revealed no alterations in the class I β-tubulin sequence; however, an α-tubulin mutation **αSer379Arg** was detected in the kα1 isotype of α-tubulin (130) (**Table 2**). Interestingly, the ability of Taxol to induce tubulin polymerization in these cells has not been impaired, suggesting that the presence of this mutation in α-tubulin is not likely to affect the binding of Taxol to its target site. The A549-Taxol resistant cells are also drug-dependent and were found to have increased microtubules dynamics (113) as well as alterations in the microtubule associated proteins MAP4 and stathmin. As a result, it has been postulated that α-tubulin, and hence the identified **αSer379Arg** mutation, may play a role in the binding of these regulatory proteins to microtubules, thus ultimately regulating microtubule dynamics.

Figure 11. Electron crystallography structure of β-tubulin with Taxol bound. *The tubulin backbone is shown in ribbons. The β-tubulin mutations identified in the two Taxol-resistant, PTX10 and PTX22 (β270 and β364) and the two Epothilone-resistant, A8 and B10 (β274 and β282) human ovarian cancer clones are highlighted. All four mutated residues cluster at the Taxol-binding site.*



Colchicine-Binding Site Tubulin Mutations

Antimitotic drugs that inhibit the binding of colchicine to tubulin appear to bind at a common site called the colchicine site. These agents are for the most part relatively simple structurally (**Figure 9**), especially when they are compared to those binding at the taxane (**Figure 8**) or the vinca binding sites (**Figure 10**). However, they are still structurally diverse. Although the prototype of this class is colchicine, it includes podophylotoxin, 2-methoxyestradiol (2ME2) and indanocine, among others. Colchicine-binding site antitubulin drugs inhibit microtubule function by causing microtubule depolymerization. Although colchicine itself is used for the treatment of gout and Mediterranean fever (105, 106), neither colchicine nor colchicine-binding site drugs are currently FDA approved for the treatment of cancer. A few colchicine-site drugs including the combretastatins and 2ME2 have been explored in clinical trials as inhibitors of tumor angiogenesis.

Acquired tubulin mutations in human cancer cells, in response to selection with colchicine-binding site drugs, were first demonstrated with indanocine as the selecting agent (131). Indanocine is a colchicine-site binding agent, as it is known to be competitive with colchicine when bound to β-tubulin (132). In this study, human T-lymphoblatoid CEM cells selected with indanocine exhibited a drug-resistant phenotype due to an acquired β-tubulin mutation (HM40 gene) in residue 350 leading to a Lys to Asn amino acid substitution. The homozygous **βLys350Asn** mutation conferred 115-fold resistance to indanocine and 30-40 fold cross-resistance to colchicine and vinblastine, respectively, while no cross-resistance was obtained with Taxol (131). In addition comparison of the parental wild-type with the β-tubulin mutant resistant clone revealed impaired indanocine-induced tubulin depolymerization in the latter, although there were no differences in the cold-induced tubulin depolymerization between the two cell lines. These results strongly suggest compromised drug binding to tubulin.

Vinca-Binding Site Tubulin Mutations

Antitubulin compounds that inhibit the binding of radiolabeled vinblastine and vincristine (**Figure 9**) to tubulin share a common binding site described as the "*Vinca* domain". The prototypes of this class are the *Vinca* alkaloids, vinblastine and vincristine, which were isolated over 40 years ago from the leaves of the periwinkle *Catharanthus roseus*. These compounds were initially studied because of their hypoglycemic activities, but were discovered to have anti-leukemic effects and cause bone marrow suppression (133, 134). Since then they have been widely used clinically for the treatment of leukemias, lymphomas, and some solid malignancies. The clinical success of these two natural products (vinblastine and vincristine) together with the elucidation of their mechanism of action on cellular microtubules, have facilitated the development of several semi-synthetic derivatives notably vindesine, vinorelbine and vinflunine, which are now used in the clinic for the treatment of cancer (102). Several other naturally occurring microtubule-interfering compounds have been identified that bind β-tubulin at the *Vinca*-domain including the marine-sponge derived halichondrins, spongistatin, dolastatins (isolated from the sea hare *Dolabella auricularia*), and cryptophycins (isolated from the blue-green algae *Nostoc sp.*) (for review see (135)). These agents depolymerize microtubules and have been explored in the treatment of cancer.

Acquired tubulin mutations in human cancer cells for the "Vinca-domain" have been reported in response to selections with vincristine (136) and the hemiasterlin analog HTI-286 (137, 138). In the first study, the human leukemia cell lines CCRF-CEM has been selected with vincristine and exhibited very high levels (22,600-fold) of drug-resistance to the selecting agent. This cell line overexpressed Pgp which accounted for a major part of the resistant phenotype, however, the Pgp modulator verapamil was able to restore only partial sensitivity to vincristine. These data led to the hypothesis that other cellular alterations might be present contributing to the overall resistant phenotype. Sequencing of β-tubulin (HM40) revealed a heterozygous state with a β-tubulin (HM40) mutation at residue **βLeu240Ile** in one allele (136). In the second study, 1A9 human ovarian carcinoma cells were selected with a synthetic analog of hemiasterlin, HTI-286. Several HTI-resistant 1A9 clones were isolated exhibiting a 57-89 fold resistance to HTI,

significant cross resistance (3-186 fold) to "Vinca-domain" binding drugs, minimal 2-4 fold cross resistance to colchicine-site drugs and collateral sensitivity to taxane-site microtubule stabilizing drugs (137). Sequencing of both α- (Kα1 isotype) and β-tubulin (HM40 isotype) in these clones revealed distinct acquired tubulin mutations in both subunits as follows: several HTI-resistant cell clones were heterozygous for β-tubulin with a mutation at **βSer172Ala**, in one allele while others harbored an α-tubulin mutation at residue **αIle384Val** in one allele or had tubulin mutations at residues **αSer165Pro** and **αArg221His** and expressed only the latter, lacking a wild type allele. In all cases the presence of these mutations has been associated with increased microtubule stability. This finding together with the location of these mutations and the cross-resistance profile of the HTI-resistant cells led to the formation of a model whereby the drug resistance phenotype is attributed to the increased microtubule stability rather than to reduced binding affinity of HTI to tubulin, although the latter has not been tested.

Table 2. Alpha and Beta Tubulin Mutations in Drug-Resistant Human Cell Lines

Cell Line and Selecting Agent	**Tubulin Mutation**	**Fold Resistance to Selecting Agent** [a]	**Fold Cross-Resistance**
Taxol			
1A9PTX10 (ovarian carcinoma)	βPhe270Val	**TAXOL** 24(115)	Docetaxel 4.2 (139) MAC321 3.4 (139) EpoA 8.9 (139) EpoB 2.8 (115) TacA 2.3 (140) TacE 4.8 (140) Vinblastine 0.5 (115) Laulimalide 1.5 (94) Noscapine 1 (141)
1A9PTX22 (ovarian carcinoma)	βAla364Thr	**TAXOL** 24 ((115)	Docetaxel 4.4 (139) MAC321 5 (139) EpoA 2.3 (139) EpoB 1.4 (115) TacA 2.1 (140) TacE 12 (140) Vinblastine 0.4 (115) Laulimalide 1.6 (94) Noscapine 1 (141)
MDA.MB231/K20T (Breast cancer)	βGlu198Gly	**TAXOL** 19 (118)	Vinblastine 1.0 (118)

KB 3-1/KB-15-PTX/099 (epidermoid carcinoma)	Asp26Glu	**TAXOL** 18 (117)	Taxotere 10 (117)
A549-T24 (non-small-cell lung cancer)	αSer379 Ser/Arg	17 (130)	Vinblastine 1.4 (130) Colchicine 1.3 (130)
Epothilones			
1A9/A8 (ovarian carcinoma)	βThr274Ile	**EPOTHILONE A (Epo A)** 40(142) 17.7(1) 55(94)	Taxol 10(142) 4.1(139) 8.0(140) 7.6(94) Docetaxel 7(142) 1.2(139) MAC321 1.6(1) EpoB 25(142) 38(94) TacA 2.9(140) TacE 4.2(140) Laulimalide 2.4(3)
1A9/B10 (ovarian carcinoma)	βArg282Gln	**EPOTHILONE B (Epo B)** 24(142)	Taxol 6.5(142) 9.4(94) Docetaxel 5(6) EpoA 57(142) 74(94) Laulimalide 3.8
A549.epoB40 (non-small-cell lung cancer)	βGln292Glu	**EPOTHILONE B (EpoB)** 95(143)	Taxol 22 (143) 21(139) Taxotere 13(9) 17(1) MAC321 10.7(1) EpoA 72 Vinblastine 0.5 Colchicine 0.6
KB-C5/0 (epidermoid carcinoma)	βThr274IPro	**EPOTHILONE A (Epo A)** 45 (144)	Epo B 8 Taxol 98 Discodermolide 1.3 Vinblastine 0.7 Demecolcine 0.7 Doxorubicine 1.6 5-FU 1.2 Paraplatine 1.2 (144)
KB-D4/40 (epidermoid carcinoma)	βThr274IPro	**EPOTHILONE A (Epo A)** 71 (144)	Epo B 9 Taxol 200 Discodermolide 0.5 Vinblastine 0.2 Demecolcine 0.5 Doxorubicine 1.2 5-FU 0.9

			Paraplatine 3.8 (144)
dEpoB30 (CCRF-CEM leukemia cells)	βAla231Ala/Thr	**dEpoB** 21 (145)	Taxol 16 EpoB No Test Vinblastine 0.28 Colchicine 0.78
dEpoB60 (CCRF-CEM leukemia cells)	βAla231Ala/Thr	**dEpoB** 60 (145)	Taxol 26 EpoB No Test Vinblastine 0.58 Colchicine 0.88
dEpoB140 (CCRF-CEM leukemia cells)	βAla231Ala/Thr	**dEpoB** 173 (145)	Taxol 7 EpoB 12 Vinblastine 0.79 Colchicine 0.88
l **dEpoB300** (CCRF-CEM leukemia cells)	βAla231Ala/Thr βGln292Gln/Glu	**dEpoB** 307 (145)	Taxol 467 EpoB 77 Vinblastine 0.25 Colchicine 0.85
Indanocine			
leukemia **CEM-178**	βLys350Asn	**INDANOCINE** 115 (131)	Taxol 1 Vinblastine 40 Colchicine 31 Fludarabine 2.3 Doxorubicin 1.9 Cytochalasin B 0.6 (131)
Vincristine			
CEM/vincristine-R (CCRF-CEM leukemia cells)	βLeu240Leu/Ile	**VINCRISTINE** 22600 (136)	