8. Microtubule binding natural substances in cancer chemotherapy

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Abstract. Microtubules constitute the major part of the cytoskeleton and play active role in cell division. Their dynamic instability and role in spindle formation during mitosis makes them an interesting target for anti-cancer drug development. Natural products are well known to be utilized for improving the human health. There are many natural products currently in use for providing cure to all kinds of diseases including cancer. Taxol and Vinblastine are examples of such natural products which interact with tubulin and are used in chemotherapy of cancer. This article briefly describes the microtubule binding natural substances and their use as anticancer agents.

1. Introduction

Natural products have shown to be the major source of anticancer drugs. In the last 25 years more than 60 % of the anticancer drugs are either natural products or have natural product origin [1]. Microtubules are one of the major components of the cytoskeleton which are essential for many cellular processes including maintenance of cell structure, protein transportation and mitosis. These are also referred to as conveyer belts inside the cell [2]. The microtubules are composed of a group of cylindrical proteins know as tubulins and perform many of their functions by binding to MAPs i.e.
microtubule associated proteins. Microtubules are directly involved in the formation of mitotic spindle which helps in segregating the replicated chromosomes towards two daughter nuclei at the end of mitosis. Involvement of microtubules in this particular cell cycle event makes them an important target in cancer chemotherapy [3]. The anticancer activity of taxanes and vinca alkaloids is attributed to their affinity and binding ability to tubulin units of the microtubules.

2. Microtubule structure and target for cancer chemotherapy

During the cell division i.e. mitosis, microtubules play an important role in segregation of chromosomes via spindle formation. Microtubules as the name suggests are the hollow tube like structures with a diameter of 15-25 nanometer and form the major part of the cytoskeleton. Their length may vary from 200 nm to 25 micrometers. This hollow structure is formed by an imperfect helix like arrangement of the protofilaments. The protofilaments in turn, are the product of end to end polymerization of the tubulin heterodimers namely alpha tubulin and beta tubulin. Polarity is another feature of the microtubule structure as during the end to end polymerization process alpha subunit of one tubulin dimer is attached to beta subunit of the other. This leads to the formation of protofilaments with beta subunits exposed at one end and alpha subunit at the other. These are designated as plus (+) and minus (−) ends respectively. In a microtubule the protofilaments bundle parallel to each other so that there is one end with beta tubulin subunit (plus end) exposed and the other with alpha (minus end) unit exposed. The minus end is capped, so that elongation occurs from the plus end [4].

The mitotic spindles are formed by attachment of GTP-tubulin to the growing end of the protofilament. The microtubules undergo rapid assembly and disassembly leading to their dynamic instability [5,6]. This dynamic instability along with their involvement in mitotic spindle formation helps in the metaphase to anaphase transition of the mitosis. This continued assembly and disassembly process in microtubules are crucial to the normal cell division and any interference in this leads to cell death via apoptosis. Usually the anti-mitotic agents arrest the metaphase to anaphase transition in mitosis. The defective spindles formed due to disturbances in dynamics of microtubules at low concentrations of the anti-mitotic agent are unable to cross the mitotic spindle checkpoint and initiate the anaphase stage. This leads to prolonged mitotic arrest and finally cell death by apoptosis.
Table 1. Diverse origin of taxane-domain binding drugs.

<table>
<thead>
<tr>
<th>ORIGIN</th>
<th>DRUG</th>
<th>SOURCE</th>
</tr>
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<tbody>
<tr>
<td>PLANT</td>
<td>Paclitaxel</td>
<td><em>Taxus brevifolia</em> (Yew tree bark)</td>
</tr>
<tr>
<td></td>
<td>Docetaxel</td>
<td><em>Taxus baccata</em> (semi-synthetic)</td>
</tr>
<tr>
<td></td>
<td>10-deacetylbaccatin III</td>
<td><em>Taxus brevifolia</em> (Yew tree leaves)</td>
</tr>
<tr>
<td>BACTERIAL</td>
<td>Epothilones</td>
<td><em>Sporangium cellulosum</em> (myxobacterium)</td>
</tr>
<tr>
<td></td>
<td>Cycloestrideptin</td>
<td><em>Streptomycetes sp.</em></td>
</tr>
<tr>
<td>MARINE</td>
<td>Discodermolide</td>
<td><em>Discoderma dissolute</em> (marine sponge)</td>
</tr>
<tr>
<td></td>
<td>Dictyostatin</td>
<td><em>Spongia</em> (marine sponge)</td>
</tr>
<tr>
<td></td>
<td>Laulimalide</td>
<td><em>Hyattella sp.</em> and <em>Fasciospongia rimosa</em> (marine sponges)</td>
</tr>
<tr>
<td></td>
<td>Peloruside</td>
<td><em>Mycale hentscheli</em> (marine sponge)</td>
</tr>
<tr>
<td>CORAL</td>
<td>Eleutherobin</td>
<td><em>Eleutherobia sp.</em> (soft coral)</td>
</tr>
<tr>
<td></td>
<td>Sarcodictyins</td>
<td><em>Sarcodictyon roseum</em> (soft coral)</td>
</tr>
</tbody>
</table>

The beta unit of the tubulin heterodimer has the priority over the alpha unit in interaction with the drugs. Its structure has been solved by electron diffraction [7]. Beta-tubulin has the binding sites for both the taxane drugs and the vinca alkaloids at different locations. The taxane drug, paclitaxel binds on two sites of the beta subunit, the N-terminal unit and the region between the amino acids 217-231 [8]. The vinca alkaloid drugs also bind to same beta subunit but in the region bound by amino acids 175 and 213 [9].
The extensively studied natural ligand of the tubulin, colchicine, binds between the two subunits and is not used clinically as anticancer drug. Another group of the natural products known as epothilones also bind to tubulin at its taxane binding site [10].

The natural products binding to the tubulin can affect its dynamics either by promoting or by inhibiting the polymerization process. Based on this general characteristic the tubulin binding natural products have been classified as under inhibitors or promoters of the tubulin polymerization.

3. Promoters of tubulin polymerization

The microtubule polymerization promotors can be broadly classified in to the taxanes and the epothilones both of which bind to same domain of the beta subunit of the tubulin heterodimer. Apart from these two classes, there are few more compounds which are known for their tubulin polymerization properties. In the following table the diverse origins of the drugs binding to taxane domain and their source has been presented.

3.1. The taxanes

Paclitaxel has been the main chemotherapeutic agent for the various types of cancers including breast, ovarian and the prostate cancer. This compound was first isolated and reported from the pacific yew tree bark in 1960 and named as Taxol [11]. Its mechanism of action was discovered in 1980s. The new and currently used generic name Paclitaxel was given when the drug was developed commercially by Bristol-Mayers Squibb and sold under the trade name Taxol. The drug is also used in chemotherapy of NSCLC in combination with Cisplatin [12].
The success of the paclitaxel led to the development of many of its analogs which are currently in clinical trials. The only analog approved in USA is the Docetaxel, which is a semi-synthetic analog and was developed in France [13]. Apart from the paclitaxel and docetaxel which are the only approved taxanes in therapeutic use there are many analogs in different phases of in clinical trials which are mostly the semi-synthetic analogs starting from 10-deacetylbaccatin III [14].

3.2. Epothilones

Epothilones belong to macrolide class of the drugs and act as microtubule stabilizers. They are produced by Myxobacterium Sorangium cellulosum and initially found to have antifungal and cytotoxic activity [15]. Later, the cytotoxic activity of these epothilones A (R = H) and B (R = Methyl) was found to be associated with mitotic arrest, which occurs via over polymerization of microtubules. Patupilone which is a natural epithilone B derivative is in phase III clinical trials by Novartis for the ovarian cancer. It has been found to be many times more effective than paclitaxel and also crosses the blood-brain barrier [16,17]. Initially another epothilone B derivative, Ixabepilone [18] has shown to be of clinical use however later it was dropped from further development.

3.3. Other compounds

Apart from the two major classes of the compounds described above with tubulin polymerization promoter activity, there are some other recently discovered compounds which have been shown to possess tubulin polymerization properties. These include Discodermolide [19], Laulimalide
[20] and Eleutherobin [21,22]. Discodermolide along with Dictyostatins was isolated from marine sponges. The sponges producing them use the microtubule toxins as part of their self-defense mechanism. Although development of the Discodermolide has been stopped there is a possibility of its derivatives to become a clinical candidate in near future. Eleutherobin was isolated [22] from corals and have similar binding properties as that of paclitaxel. A total synthesis has been developed for this molecule, although it is not yet in clinical trials [23]. Laulimalide which also stabilizes the microtubules has a different binding site on tubulin in contrast to Paclitaxel. It has potential to kill paclitaxel and epothilone resistant cells and a total synthesis for this molecule has also been reported.
4. Tubulin polymerization inhibitors

Vinca alkaloids constitute the major class of the compounds that inhibit the polymerization of tubulins. Other important compounds in this category include the Combretastatins, Dolastatins, Noscapine analogs, Hemisterlin and Rhizoxins. The Table 2 gives a summary of the compounds with tubulin destabilizing activity along with their natural origin and chemical nature.

Table 2. Vinca-domain binding drugs of diverse origin.

<table>
<thead>
<tr>
<th>Name of the drug</th>
<th>Source</th>
<th>Chemical nature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinblastine</td>
<td></td>
<td>Alkaloids</td>
</tr>
<tr>
<td>Vincristine</td>
<td><em>Catharanthus roseus</em></td>
<td><em>(Vinca rosea)</em> and analogs</td>
</tr>
<tr>
<td>Vinorelbine</td>
<td></td>
<td></td>
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<tr>
<td>Vinflunine</td>
<td></td>
<td></td>
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<tr>
<td>Vindesine</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maytansinoids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maytansine</td>
<td><em>Maytenus ovatus</em></td>
<td>Macrolide</td>
</tr>
<tr>
<td>Ansamitocins</td>
<td><em>Nocardia</em></td>
<td>Macrolide</td>
</tr>
<tr>
<td><strong>Marine origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolastatin 10</td>
<td><em>Dolabella auricularia</em></td>
<td>Pseudo peptide</td>
</tr>
<tr>
<td>Dolastatin 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halichondrin</td>
<td><em>Halichondira okadai Kadota</em></td>
<td>Lactone polyether</td>
</tr>
<tr>
<td>Spongistatin 1</td>
<td><em>Hyrtios altum</em></td>
<td>Macrocyclic lactone</td>
</tr>
<tr>
<td><strong>Fungal origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizoxin</td>
<td><em>Rhizopus chinensis</em></td>
<td>Macrocyclic lactone</td>
</tr>
<tr>
<td>Phomopsin A</td>
<td><em>Phomopsis leptostomiformis</em></td>
<td>Peptide</td>
</tr>
<tr>
<td>Ustiloxin</td>
<td><em>Ustilaginoidea virens</em></td>
<td>Peptide</td>
</tr>
</tbody>
</table>
4.1. Vinca alkaloids

The vinca alkaloids vinblastine and vincristine were the first natural products to enter in the clinical use for cancer chemotherapy. These compounds were isolated by two different research groups in late 1950’s and early 1960’s from Madagscar periwinkle known as *Vinca rosea* or *Catharanthus roseus* [24].

One of the groups working on them was interested in finding a substance affecting the blood glucose levels. However, at the same time they also noticed the effect of the extract on the white blood cell counts. This lead to the discovery of its antileukemic activity and finally the isolation and structure elucidation of vincaleukoblastin which was later shortened to vinblastine [25].

![Vinblastine](image)

The vinca groups of alkaloids binding to the beta subunit of tubulin are constituted by several closely related compounds. These include vincristine, vindesine, vinorelbine and vinflumine, which are the semisynthetic vinca alkaloids. Vinblastine and vincristine are in clinical use as anticancer drugs since last 50 years. They are also used in combination therapy of acute leukemias and lymphomas, bladder and breast cancers [26].

4.2. Combretastatins and derivatives

Although the vinca alkaloids are the only tubulin polymerization inhibitor compounds which are in clinical use, there are several other groups of compounds which bind to same domain of the tubulin and have similar mechanism of action. Many of these analogs are in advanced stages of clinical trials e.g. Combretastatins, which were isolated from the root bark of *Combretum caffrum* [27].
They are well-known as antimitotic agents and Combretastatin A2 (CA2) & Combretastatin A4 (CA4) are the most potent members of this family. CA4 is highly cytotoxic than its tubulin destabilizing activity [28]. The phosphorylated CA4 known as CA4P has anti-angiogenic properties via the disruption of the endothelial cytoskeleton [29]. This compound is in phase III trials for treatment of cervical, colorectal, NSCLC, prostate and ovarian cancers [30,31].

### 4.3. Dolastatins

Pettit isolated Dolastatin 10 from *Dolabella auricularia*, the most potent member of a big family of dolastatins [32]. It has a distinct binding site on tubulin where usual antimitotic peptides bind [33]. In 1990 it entered the clinical trials by NCI for solid tumor treatments. Another peptide, Dolastatin 15 is also as potent as Dolastatin 10 but in contrast to the later, it is not involved in the nucleotide exchange inhibition and aggregation induction. One of the Dolastatin 15 derivatives is in phase II clinical trials [34].

### 4.4. Noscapinoids

The phthalideisoquinoline alkaloid from *Papaver somniferum*, Noscapine is in medicinal use since long for its antitussive activity [35]. Currently this molecule is in phase I-II clinical trials for the treatment of multiple myeloma. Noscapine and its derivatives are different from other microtubule binding drugs in the fact that they keep the total polymer mass of the tubulin unaltered [36].
Stoichiometric binding of Noscapine induces a conformational change in tubulin and interrupts the cell cycle in mitosis. It inhibits the dynamic instability of tubulins by extending the relaxation time. Several analogs of the parent molecule have been prepared and evaluated against diverse cancer cell lines. It has been concluded that noscapinoids are the gentlest molecules involved in the microtubule dynamics creating mitosis checkpoints without any significant toxicity profile [37].

4.5. Eribulin and halichondrins

These complex natural products of marine origin were isolated from western pacific sponge *Halichondria okadai* and also from *Axinell sp* [38,39]. This class of molecules have shown to be highly cytotoxic specially the Halichondrin B and homohalichondrin. These compounds were shown to bind to tubulin and inhibit their polymerization. They have shown sub-nanomolar activity in NCI’s 60 cell anti-cancer screening panel along with promising activity in many animal models [39]. The attempts towards the total synthesis of Halichondrin B resulted in the discovery of Eribulin [40]. Similar to its parent, it also inhibits tubulin polymerization and is currently in phase III clinical trials for several cancer types [41].
4.6. Hemiasterlin

Hemiasterlin is a tripeptide of marine origin. It was first isolated from *Hemiasterella minor* and found to be active against murine leukemia cell lines [42]. Later, its antitubulin and antimitotic activity was discovered by Anderson [43]. The phenyl alanine derivative of the parent compound, HTI-286 [44] has been found to be more potent and more synthetically accessible. Both these molecules are in clinical trials [45].

![Hemiasterlin molecule](image)

4.7. Rhizoxin

Rhizoxin was isolated from a plant pathogenic fungus *Rhizopus chinensis* and was discovered to be inhibitor of tubulin polymerization [46]. It is a macrocyclic lactone, although very similar to maytansine [47], it is comparatively more potent against human and murine tumor cells. It has been synthesized and gone through the clinical trials. The molecule is yet to be approved for clinical use.

![Rhizoxin molecule](image)
5. Challenges and future prospects

Since last two decades the importance of tubulin dynamics as a target for anticancer drug development has been increased significantly. The established tubulin interactive drugs include the two vinca alkaloids and the taxens, paclitaxel and docetaxel. Epothilones have just been made available clinically. There are many candidates in phase II-III trials as described earlier. It is now well know that almost all tubulin interactive agents are the natural products. The supplies of these compounds for clinical use in near future should be guaranteed. In future apart from the discovery of the new tubulin interactive agents, the development of the novel noscapinoids, taxanes, epothilones and other compounds will continue towards finding the new and improved drug candidates. Next, the targeted drug delivery approach would augment the current situation e.g. the use of nanoparticles for the targeted delivery of Noscapine is under investigation [49]. Design of simpler synthetic compounds taking the clue from molecular modeling studies and better understanding of the tubulin interactions with known molecules would also play a major role in development of new and improved anticancer agents in future [50].

References