Pharm Res
DOI 10.1007/s11095-012-0886-2

EDITORIAL

# Drugs Targeting Tubulin Polymerization

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Received: 10 September 2012 / Accepted: 10 September 2012


Tubulin polymerization represents a fertile target for anti-cancer drugs. Currently, there are several Food and Drug Administration (FDA)-approved agents for this target, such as paclitaxel, docetaxel, and epothilones. While these drugs enjoy wide success clinically in treating patients with various types of cancers, the development of drug resistance upon prolonged use and undesired toxicities, especially neurotoxicity, present limitations for their clinical efficacy.

Cancer cells develop resistance to anti-mitotic drugs in many ways. They can significantly up-regulate efflux pumps to reduce effective drug concentrations or frequently develop mutations to weaken or prevent the binding of an anti-mitotic drug to the tubulin. Extensive research efforts have been made in both academia and industry to understand the underlying mechanisms of drug resistance and develop new strategies to overcome the resistance by fine-tuning existing anti-mitotic drugs or discovering new drugs that may have improved efficacy. This is a very active and important field of research. In this theme issue, contributions from experts working in a number of areas on this target are presented.

The first three research papers in this theme issue describe efforts to understand possible resistant mechanisms to anti-mitotic drugs. Yin *et al.* employed a random mutagenesis strategy to generate sets of β1-tubulin mutations and tested their resistance to paclitaxel. This approach rapidly enlarged the spectrum of known β1-tubulin mutations. Very interestingly, they found that several of the β1-tubulin mutations they generated conferring paclitaxel resistance have also been reported in patients with a variety of neuronal disorders, although those mutations occurred in β2 or β3 instead of β1 tubulins. The authors suggested that because β1 tubulin has very low abundance in the brain, neurons may find other abundant isotypes to generate these resistant mutations for paclitaxel resistance. Tuszynski *et al.* studied paclitaxel drug resistance from a different angle. The yew tree is the main source of paclitaxel, and, by definition, the yew tree is resistant to paclitaxel. It is very interesting to understand why the yew tree is immune to this highly toxic effect. Understanding such resistance at the molecular level may help us understand the development of drug resistance mechanisms clinically. The authors employed molecular modeling approaches to build a homology model of yew tree tubulin, compared the binding of paclitaxel in yew tree with that of human tubulin, and identified a number of residues in the high-affinity region for paclitaxel binding that are different between yew tree and human tubulin. These unique residues in the yew tree tubulin reduce the binding energy for paclitaxel and are likely responsible for its resistance to paclitaxel. This study could provide guidance to identify similar changes in cancer patients or, ultimately, to predict possible drug resistance.

Kanakkanthara *et al.* aimed to understand acquired drug resistance to another set of anti-mitotic agents, Peloruside A (PLA) and Laulimalide (LAU). Similar to paclitaxel and its analogs, PLA and LAU produce their anticancer effects by stabilizing microtubule formation, but they are believed to bind to tubulin at a different site than paclitaxel. Using a proteomics approach, the authors identified levels for several proteins that have been significantly altered between resistant cells and their sensitive parental cells. In particular, downregulation of vimentin was found to strongly contribute to the resistance to PLA and LAU.

The next four research papers report on recent efforts in developing new small molecular anti-mitotic agents targeting the colchicine binding site. Unlike other currently known binding sites in tubulin, there is no FDA-approved drug interacting with this binding site. Currently, all existing

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Published online: 28 September 2012

anti-tubulin drugs have poor aqueous solubility and poor oral bioavailability and therefore have to be administered intravenously. Developing an orally available agent with comparable efficacy to existing anti-tubulin drugs, such as docetaxl, while effectively overcoming clinically relevant multidrug resistance associated with existing anti-tubulin drugs would significantly benefit cancer patients. Since structures for molecules interacting with the colchicine binding site are relatively simple, they are amicable to extensive structural optimizations to improve their drug-like properties. The relatively simple structure features also mean a variety of small molecules may bind to this binding site; therefore, diverse scaffolds have been reported to be colchicine site ligands.

Gangjee *et al.* describe their efforts in designing a novel class of water-soluble small molecules interacting with the colchicine binding site based on the pyrrolo[3,2-d]pyrimidine scaffold. They report their rational design, synthesis, and *in vitro* biological testing. Many of these compounds show low nanomolar activity against a variety of cancer cell lines, as indicated by the results from NCI-60 assays. More importantly, these compounds are water soluble, which may offer a great advantage over existing anti-mitotic agents which are usually hydrophobic and have to rely on special formulations using surfactants (*e.g.* Cremophor EL). These compounds will serve as leads for further optimization.

The papers by Wang *et al.* and Li *et al.* describe efforts in designing potent small molecules targeting the colchicine binding site. Wang and his colleagues report new analogs based on the *a*ryl-4-*b*enzol-*i*midazoles (ABI) scaffold that can overcome several clinically relevant, ABC-transporter-mediated multidrug resistant mechanisms. They also showed in a mouse model that these compounds at a relatively low dose have comparable efficacy with high dose dacarbazine against melanoma tumors. These two papers present extensive preclinical studies for two orally bioavailable, highly potent small molecules targeting the colchicine binding site. These compounds possess good *in vitro* drug-like properties and acceptable *in vivo* pharmacokinetic properties. They are significantly more potent in reducing paclitaxel- and docetaxel-resistant prostate tumors *in vivo*. Collectively, these results suggest that small molecular compounds may have therapeutic potential for oral use in paclitaxel- or docetaxel-refractory cancers.

While they usually have high potency against cell proliferation, most tubulin targeting drugs have limited aqueous solubility and poor selectivity against cancer cells. Drug formulation and delivery using non-toxic, bio-degradable, polymeric nanoparticles is a promising strategy to overcome some of these limitations. The research paper by Mundra and colleagues describes their efforts in formulating a small molecule tubulin inhibitor using polyester/polycarbonate based nanoparticles. Using melanoma as the cancer model, their results suggest that bio-degradable polymeric nanoparticles could be an excellent platform to deliver different hydrophobic drugs to cancer cells.

In addition to the seven research papers, this theme issue also contains three review papers covering three distinct areas related to anti-tubulin drugs. Lu *et al.* present a comprehensive review on colchicine binding site inhibitors (CBSIs) over the past 10 years. It is clear from this review that small molecules with diverse scaffolds can interact with the colchicine binding site in tubulin, and computer modeling has been used to identify the critical pharmacophores needed for the binding. Overall, this is a very active research field, with hundreds of highly potent compounds reported in the recent literature. The ongoing significant research efforts from both academia and industry will likely to produce the first FDA-approved anticancer drug targeting the colchicine site in tubulin in the near future.

Gajewski *et al.* review recent reports on binding sites, binding modes, and binding affinities for Peloruside, Laulimalide and Noscapine. Since the crystal structures for these new molecules in complex with tubulin are not available, their exact mechanisms of actions and detailed atomic interactions to tubulin remain to be elucidated. The authors reviewed recent reports using molecular modeling approaches to help address these questions. They also discussed the efforts made in creating derivatives and analogues of these parent compounds aimed at improving their pharmacological profiles.

Stack and Walsh contributed a review of the delivery of tubulin targeting agents through judicious conjugation with antibodies. They examine reported evidence supporting the hypothesis that properly formed drug–antibody conjugates may selectively deliver the drug to tumor sites, therefore reducing toxicity often associated with systematic drug administration. In particular, they examine strategies in selecting different linkers connecting anti-tubulin drugs to various antibodies and summarize drug-antibody conjugates in preclinical development as well as in clinical trials. Even though there are still significant challenges, attaching a potent anti-tubulin agent to a well-validated antibody in a specific type of cancer *via* an optimal linker will likely improve tumor selectivity, overcome drug resistance, reduce side effects, and ultimately provide significantly better therapeutic efficacy.

Research involving tubulin-targeting drugs is a vast and fast-advancing field, spanning virtually all basic and applied scientific disciplines. A lot of challenges remain. Due to the ubiquitous presence of tubulin in human tissues and organs, toxicity and side effects remain the biggest challenge. While there are many isotypes of tubulin proteins, attempts to determine differentially expressed isotypes between tumors and healthy cells have been challenging. In addition, different cancer cells often have different isotype distributions, the overall homologs of tubulin isotypes are high, and often a

single mutation in the binding pocket can confer strong drug resistance. Available crystal structures of a drug in complex with tubulin protein have poor resolution, making detailed mechanistic studies and structure-based drug design difficult. Nevertheless, significant advancements have been made in recent years. There is no doubt that tubulin targeting agents will continue to play a significant role in effective treatment for many cancer types. We hope the papers contained in this theme issue illustrate some of recent important advances for the readers of *Pharmaceutical Research*.
The guest editor would like to extend his sincerest gratitude to all the contributors and reviewers for the papers published in this issue. The help and advice from the editor's office, especially from Ms. Rachel Lucke and Dr. Ram Mahato during this process are greatly appreciated.

## INTERVIEW WITH DR. WEI LI

*What do you think holds the key to your success as a pharmaceutical scientist?*

There are a lot of factors. The most important factor is that I am fortunate to work with very talented graduate students and excellent collaborators and mentors. In addition, while I was not formally trained as a pharmaceutical scientist, the rigorous training I received at the University of Science and Technology in China, the Chinese Academy of Sciences, and Columbia University has been very helpful.

*What do you consider to be your key research accomplishments?*

As a medicinal chemist, I consider the licensing of new small molecules developed in the lab to companies for further commercial development into potential therapeutic agents a key research accomplishment.

*What was the turning point in your career?*

The turning point of my career was when I began my independent research career in the college of pharmacy at the University of Tennessee Health Science Center in 2004. I have excellent collaborators, and there have been many opportunities to develop my career.

*Which individuals have most influenced your research career?*

I was fortunate to have met and worked with many outstanding mentors and collaborators in my career, but three in particular stand out. In the earlier years of my research career in the Turro's group at Columbia University, I had the opportunity to work with Professor Nick Turro and Dr. Xuegong Lei, who was a research scientist in the group. I learned how to define a problem and try to find ways to solve it. When I moved to University of Tennessee Health Science Center, I was fortunate to learn and work with Dr. Duane Miller, who is an exceptional medicinal chemist.

*What is the key to developing successful collaborative relationships between pharmaceutical/medicinal chemists like you and more applied pharmaceutical/formulation scientists who can help in product development?*

Like in any type of collaboration, the key to a successful collaboration between medicinal chemists and more applied pharmaceutical/formulation scientists requires everyone to genuinely work toward the goal. Collaborators have different areas of expertise, and it is understandable they may have different goals for working in a particular project. For example, while medical chemists like to make many analogs based on a lead compound with the aim to understand structure-activity relationships in order to further optimize the lead compounds, more applied pharmaceutical/formulation scientists often prefer to focus working on one particular compound. Therefore, clear communications to ensure every party understands the work involved and the benefits from working on the project will help to make a strong team for a productive collaboration.

*What is your philosophy of educating graduate students?*

I like to see graduate students receive extensive training and have a reasonably broad knowledge base in related fields of study. With the fast-changing biomedical research field, it is not uncommon for a graduate student to develop a career that is different (although often related) from what he/she was initially trained as a graduate student. Having a broad knowledge base and being flexible is very helpful in today's world. Good communication skills, including the ability to write very well, are also very important, especially for graduate students interested in future academic positions. Finally, nothing can substitute working hard. By definition, students entering into graduate programs are all very intelligent, so a graduate student working for 50 h per week will likely to achieve more than a graduate student working for 40 h or less per week.

*What is the place for collaboration with industry in academia?*

I believe the collaboration between academia and industry is very important for two main reasons. First, as a medicinal chemist whose interest is to discover potential new therapeutic agents, having collaboration with industry will help us to calibrate important factors an industry looks for before taking our patented compounds. In academia, we can only move to a certain point (*i.e.*, *in vivo* testing with small animals)

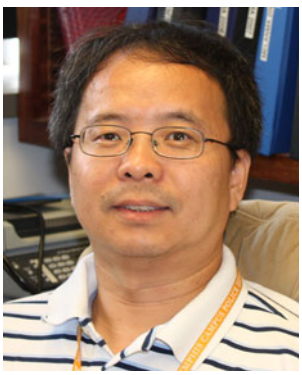
at which we would like an industry partner to take over for further commercial development. If no commercial partner was interested in developing a patented class of compounds, the impact of the patent would be limited. After all, most of our research is supported by taxpayer money, and the research will have more impact on patients and society if a potential drug candidate can be developed from the supported research. Second, as the funding from NIH to support drug discovery research is becoming limited, collaboration with industry may provide an alternative source of funding to support research.

*Pharmaceutical scientists are faced with the dilemma of having to publish in biomedical, chemistry, or basic science journals. Does this mean cutting-edge science will not likely be featured in journals like* Pharmaceutical Research*?*

I don't think so, especially for researchers working in drug discovery area. In this area, chemistry and biology are highly integrated. The selection of a journal to publish research work is dictated by the contents of the manuscript. While there are certainly many more focused journals available, *Pharmaceutical Research* encompasses different sections covering broad research areas of interest to pharmaceutical scientists, and it is an excellent journal to publish cutting-edge sciences. In fact, papers in this theme issue are examples of the broad coverage in *Pharmaceutical Research* from molecular modeling and medicinal chemistry all the way to pharmacokinetics and drug delivery studies.

*What are the challenges for drug discovery and development, and how can they be overcome?*

As we all know, successfully putting a drug to the market is a long, expensive, and highly risky process. It is especially challenging nowadays since the relatively easy targets, or "low hanging fruits," have been taken. The stringent safety and efficacy requirements also present significant challenges. However, with the advancement of biology and in-depth understanding of disease mechanisms, new drug targets are constantly being discovered, and some of them have resulted in amazing medicines that were not possible before. An example is the development of vemurafenib that was approved last year. This drug is highly efficacious for melanoma patients having the B-raf V600E mutation. While it is very difficult to completely overcome the intrinsic challenges associated with drug discovery and development, more targeted approaches with well-validated mechanisms will likely help to alleviate these obstacles.



**Dr. Wei Li** is an Associate Professor in the Department of Pharmaceutical Sciences, College of Pharmacy, the University of Tennessee Health Science Center. He obtained his B.S. degree in chemical physics from the University of Science and Technology of China in 1992. After 2 years of graduate study in Dalian Institute of Chemical Physics, the Chinese Academy of Sciences, he went to New York City for his graduate study with Professor Nick Turro at Columbia University in 1994. Upon completion of his Ph.D. study in 1999, he took a position as the NMR facility manager in his current institution followed by a tenure-track faculty appointment in 2004. Currently, research in his lab broadly focuses on discovering new tubulin inhibitors binding to the colchicine binding site as potential agents against malignant melanoma and developing new synthetic, noncalcemic vitamin D3 analogs as anti-inflammation agents. His lab also works on applications of high-resolution magic angle spinning NMR (HRMAS NMR) techniques for studies involving intact cells, tissues, or the characterization of chemically modified nanostructures. He has published over 70 peer-reviewed papers and is an inventor of several pending patents.