COMMENTARY

A previously undescribed tubulin binder

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Microtubules (MTs) are key components of the cytoskeleton for all eukaryotes and are vital for cellular processes such as mitotic spindle formation, intracellular transport, vesicle formation, cellular signaling, and migration. These functions depend on the dynamic nature of MTs, which arises from the polymerization and depolymerization events of individual heterodimers, made up of α- and β-tubulin subunits (1). Strict control and appropriate regulation of the equilibrium between free tubulin and MTs is therefore critical for cell viability and has provided the basis for the development of several drugs used in treating cancer (2), autoimmune diseases (3), and neurological diseases (4). Understanding how the drugs bind to tubulin has proven invaluable not only in elucidating how they function, but also for designing new lead agents. In PNAS, Prota et al. add significant new insights into this field by defining a site within tubulin that is able to bind clinically relevant anticancer drugs in a manner that has not been previously described (5).

Structurally, MTs are quite complex, consisting of parallel protofilaments formed from α,β-tubulin heterodimers (Fig. 1*A*). Their organization is highly dynamic, rapidly fluctuating between periods of growth and shortening. Polymerization is linked to GTP hydrolysis at the terminal tubulin β-subunit, and disassociation releases GDP tubulin (6). Because maintaining a balance between the tubulin dimers and the MTs is critical for cell viability, and many small molecules and proteins act through equilibrium (2, 7). Major drugs, consisting of the vinca alkaloids and taxoids, have long been used in chemotherapy, with considerable success in cancer management (8). The field is rapidly evolving, with new antimitotic drugs and antibody drug conjugates comprised of antitubulin agents such as monomethylauristatin E (9) and a maytansinoid known as DM1 (10).

The majority of MT-binding drugs are, or have been, derived from natural products, which have evolved to target eukaryotic tubulin with high affinities. Typically, they have been classified as either stabilizers or destabilizers depending on their effect on MT mass (8). Stabilizing agents promote tubulin assembly by increasing lateral protofilament interactions (11–13), whereas destabilizing agents inhibit MT polymerization by promoting a curved conformation or inhibiting a straightened conformation necessary for proper MT formation (14, 15). Each group of compounds is extremely structurally diverse and has historically been defined by their binding site as determined by in vitro competition assays. There are currently four structurally distinct and well-characterized regions on MT where drugs have been known to bind (Fig. 1*A*): the taxoid site on the luminal face β-subunit (11, 13), the laulimalide/peloruside site on the external face β-subunit (12), the colchicine site at the β-tubulin subunit intradimer interface (14), and the vinca site at α,β-heterodimer interface (15).

The vinca site is a complex drug-binding site located between two heterodimers (Fig. 1*B*). MT-binding drugs to this site induce heterodimer aggregation, which at high concentrations causes the formation of curved and spiral tubulin assemblies (16). Vinca site binders therefore pose two individual rate constants, binding to the β subunit and induction of heterodimer aggregation (17, 18). A number of different chemical moieties can bind the vinca site, and structural characterization has further subdivided this site into a vinca site and a partially overlapping peptide site that terminates above the bound GDP (19). Although classified as vinca site binders, there has been substantial uncertainty over the actual binding location of the well-known maytansine and rhizoxin antimitotics (20). Both compounds interfere with vinblastine binding, but neither causes the characteristic spiraling or donuts that arise when tubulin is treated with vinca alkaloids. Furthermore, a newer molecule, PM060184, was shown recently to interfere with vinblastine binding, but it was noted that the interaction was not that of a traditional vinca site compound (21).

Using X-ray crystallography to structurally characterize a multicomponent bovine brain tubulin complex, Prota et al. describe a novel tubulin-binding site on the β-subunit capable of binding maytansine, rhizoxin, and PM060104 (5) (Fig. 1*B*).The binding site of these compounds is in a structurally distinct location to that of the vinca site. Although these compounds are chemically unrelated, their generally shared site of interaction validates this location as a bona fide novel binding site for MT destabilizing compounds. Furthermore, the structures shown in this position shed light on the molecular mechanism by which they destabilize MTs and also how this class of molecule can noncompetitively compete with vinca site compounds to inhibit the spiraling potential. Unlike the vinca site destabilizers, which induce curved

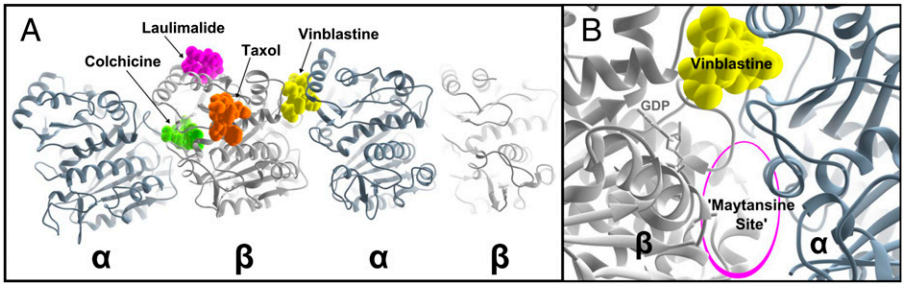

Fig. 1. Structural overview of MT-binding sites on tubulin heterodimers. (*A*) Two αβ heterodimers are displayed in the conformation seen in bovine brain tubulin crystal structures (5). The α and β subunits are colored in slate and gray, respectively. Representative MT-binding compounds are depicted as space-filling models, shown in their approximate binding positions. (*B*) Detail of the vinca site structural features. The bound GDP molecule is shown in stick representation for reference. The approximate location of the maytansine site described by Prota et al. (5) is located below the bound GDP.

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aggregation, binding at the maytansine site blocks subunit addition. In this manner, maytansine site binders either sequester soluble tubulin in an unpolymerizable form or poison the ends of growing MTs. When assayed in competition with the vinca compounds, the maytansine site binding compounds partially compete by blocking heterodimer addition and subsequent formation of the complete high-affinity vinca site.

Although MT binders have been hugely successful as anticancer drugs, a number of side effects and resistance mechanisms are associated with their use (2, 8). As a result, new compounds and targeting technologies are needed. The structures presented in this paper illustrate an additional site within the tubulin structure that can be probed for the development of drugs that will complement existing therapeutics and potentially be used in new drug combinations (8). The results illustrate that there is much uncharted territory within tubulin that may be exploitable for novel drug discovery.

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