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Review

What tubulin drugs tell us about microtubule structure and dynamics

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ABSTRACT

A wide range of small molecules, including alkaloids, macrolides and peptides, bind to tubulin and disturb microtubule assembly dynamics. Some agents inhibit assembly, others inhibit disassembly. The binding sites of drugs that stabilize microtubules are discussed in relation to the properties of microtubule associated proteins. The activities of assembly inhibitors are discussed in relation to different nucleotide states of tubulin family protein structures.

Contents

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1. Introduction

Microtubules, like other cytomotive protein filaments, produce motility simply by assembling and disassembling multi-subunit polymers. Assembly is favoured when GTP is bound, disassembly when GDP is bound. The main mechanisms by which polar polymers produce movement are known as "treadmilling" and "dynamic instability".

Treadmilling was predicted by Wegner [see 1] for eukaryotic actin; ATP hydrolysis allows structural differences between the two ends of a filament, affecting the on-rates, off-rates and critical concentrations of free subunits. If the critical concentration at one end is higher than at the other, an equilibrium concentration of free subunits promotes continuous addition to one end (the "plus" end) and continuous loss from the other ("minus") end. Dynamic instability was discovered [2] for microtubules reassembled in vitro. Mitchison and Kirschner suggested that switches in behaviour at a microtubule end reflect the presence or the absence of a stabilizing 'cap' of GTP-bound tubulin. If the concentration of free subunits is sufficient, a dynamic filament may continue to grow at either end so long as the end is stabilized by the presence of a cap but if unstable subunits containing GDP are exposed, a "catastrophe" leads to rapid loss. The filament may shrink completely or be rescued and regrow. This stochastic behaviour allows each end of a dynamic microtubule to grow or shrink independently, even in a constant concentration of free tubulin, and thus search wide regions of cytoplasm until an end contacts a stabilising target, such as the kinetochore of a condensed chromosome.

Some prokaryotic members of the actin superfamily naturally exhibit dynamic instability, rather than treadmilling; some members of the tubulin/FtsZ superfamily steadily treadmill, rather than being unstable at both ends [3]. Moreover, eukaryotic F-actin in cells or under cell-like in vitro conditions can undergo catastrophic bursts of disassembly under the influence of accessory proteins, while microtubules with both ends under control, like kinetochore microtubules in the mitotic spindle, can treadmill [4]. Interphase microtubules are stabilized by structural accessory proteins or post-translational modification of tubulin. Complementary controls on free tubulin concentration occur by regulating transcription and sequestering surplus subunits. Thus, in vivo, microtubule behaviour is under strict control [5,6]. Being essential for cell growth and also being very highly conserved in amino acid sequence, tubulin is a prime target for attacking cells and organisms. The careful balance in cells can be disturbed by peptides or small molecules that bind to the subunits, modifying their interactions. Natural agents have the hostile purpose of poisoning the growth of other organisms. But medically the roles of microtubules make them an important target for drugs to control diseases [7–9]. This chapter focuses on structural aspects of tubulin-binding drugs and the ways they interfere with microtubule dynamics.

2. Outline of tubulin structure

The structure of αβ-tubulin heterodimers modeled in ~3.5-Å resolution maps (Fig. 1) [10–13] shows similar monomers, each with two globular domains (the N-terminal "GTPase domain" – green in Fig. 1 – and the smaller "activation domain" – blue) separated by a central helix (H7 – yellow). There is also a C-terminal domain (helices H11 and H12 in pink). The subunits assemble as polar protofilaments, with a binding site for guanosine nucleotide sandwiched between the GTPase domain of one monomer and the activation domain of the next. GTP is bound to each tubulin monomer at loops T1–T5 of the GTPase domain. Upon microtubule assembly, GTP bound to β-tubulin is contacted by loop T7 and helix H8 of the α-tubulin activation domain, which promotes hydrolysis of the nucleotide to GDP and inorganic phosphate [14–17]. GTP bound to α-tubulin is permanently trapped in the heterodimer, and is never hydrolysed because β-tubulin has lysine at the end of helix H8, instead of an acidic residue (like α-tubulin E254).

2.1. Conformational changes during assembly and disassembly

Protofilaments in a microtubule run straight but during disassembly they separate and curve outwards to form rings. Crystal structures of the curved state (Fig. 1b,c) contain complexes of the sequestering protein RB3/stathmin interacting with two tubulin heterodimers [10–12]. The amino-terminal part of stathmin caps the bottom of one α-tubulin per tetramer, preventing polymerization. Changes compared with the straight conformation (Fig. 1a), resolved in Zn-induced 2D sheets [13], include a small axial displacement of helix H7 plus a slight rotation between the GTPase and activation domains, while contacts between monomers are maintained by local movements of helices H6 and H8 and loop T5 [10]. No high-resolution structure shows β-tubulin with a nucleotide other than GDP in the exchangeable site; however, the structure of α-tubulin with GTP bound is closely superimposable on that of β-tubulin with GDP. The similarity includes equal ~12° bends at all interfaces. Furthermore, crystal structures of homologous proteins, such as γ-tubulin and bacterial FtsZ, show no obvious changes in subunit structure whether GTP or GDP sits on the GTPase domain [18,19].

Because no correlation between bound nucleotide and protein conformation was apparent, subunits were proposed to convert stochastically between curved and straight conformations but preferentially appear curved unless constrained to be straight [19–21]. In a microtubule, lateral contacts