Investigating the mechanism of action of most successful anti-cancer drugs Taxol and Taxane group of drugs in real time at the level of single filaments

Microtubules, the dynamic polymers of eukaryotic cytoskeleton, play important roles in several cellular functions including cell division, cell differentiation, cell migration, intracellular trafficking and cell signalling cascades. Microtubules are polymers of tubulin dimers, and conformational transitions in the microtubule lattice drive microtubule dynamic instability and affect various aspects of microtubule function. Several clinically used anticancer drugs target microtubules network and block the cell cycle progression in mitosis, which eventually activates the cell death program. Microtubule targeting drugs are found to be very successful in cancer chemotherapy. However, the exact nature of these transitions and their modulation by anti-cancer drugs such as Taxol and epothilone, which can stabilize microtubules but also perturb their growth, are poorly understood.

In the work, using invitro reconstitution assays (Figure 1), I directly visualized the action of fluorescent Taxol and epothilone derivatives on microtubule dynamics (Figure 2).

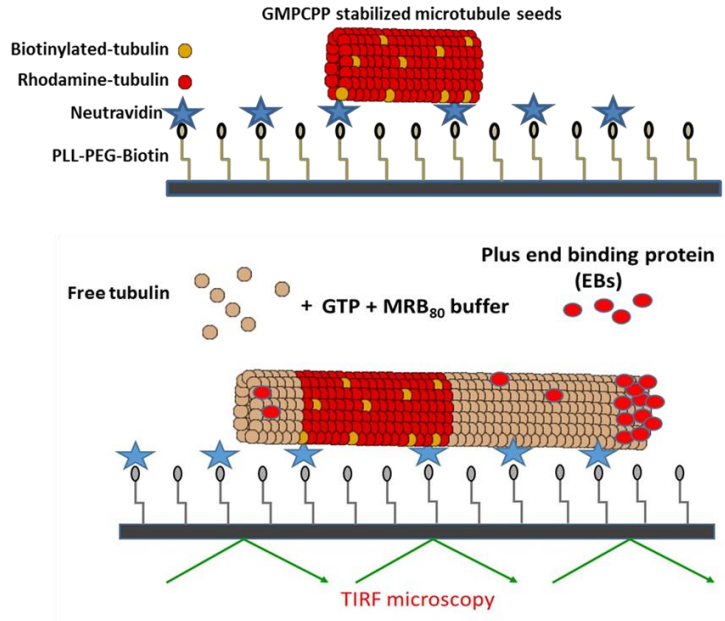

Figure 1: invitro reconstitution assay for microtubule dynamics

I showed that microtubules can transition to a state that triggers cooperative drug binding to form regions with altered lattice conformation. Such regions emerge at growing microtubule ends that are in a pre-catastrophe state and inhibit microtubule growth and shortening (Figure 3).

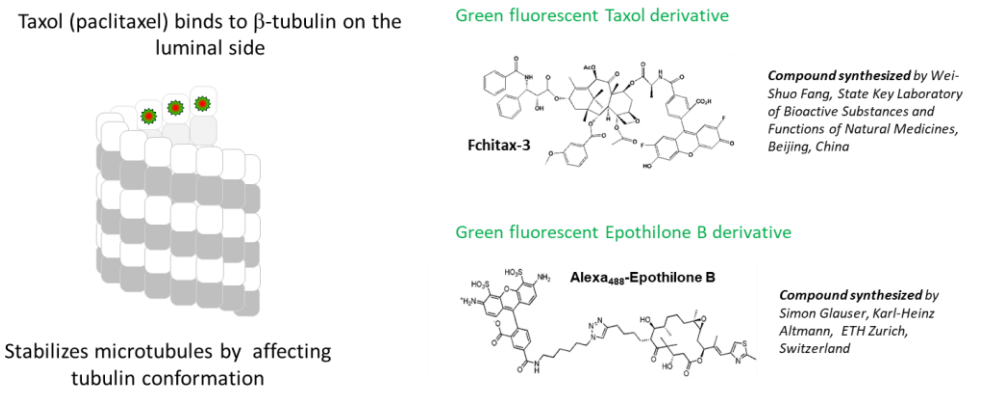

Figure 2: Microtubule-stabilizing agents: Fluorescent derivative of taxanes

Electron microscopy and in vitro dynamics data indicated that taxane accumulation zones represent incomplete tubes that can persist, incorporate tubulin dimers and repeatedly induce microtubule rescues (Figure 3).

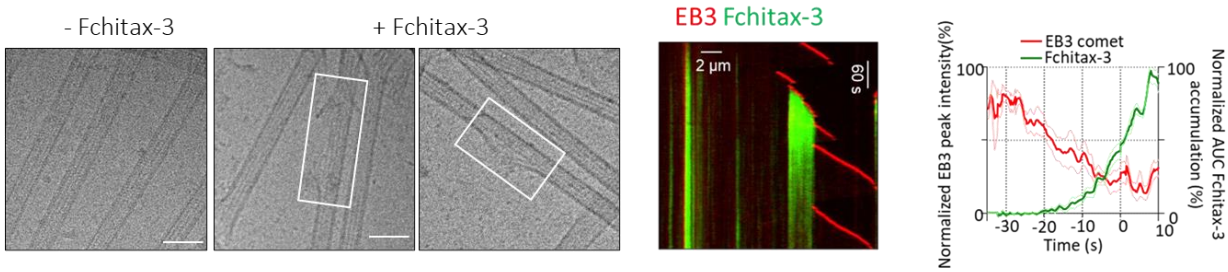

Figure 3: Taxane induces microtubule lattice defects in a cooperative manner

Thus, taxanes modulate the material properties of microtubules by converting destabilized growing microtubule ends into regions resistant to depolymerization **(Rai et al., 2020, Nature Materials).**

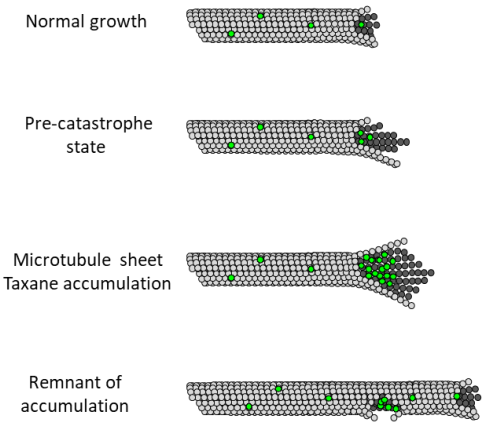

Figure 4: Lattice defects caused by mismatch in protofilament number persist and induce catastrophes

In addition, very recently, I showed that such drug-induced defects led to frequent catastrophes and induced protofilament number mismatch.

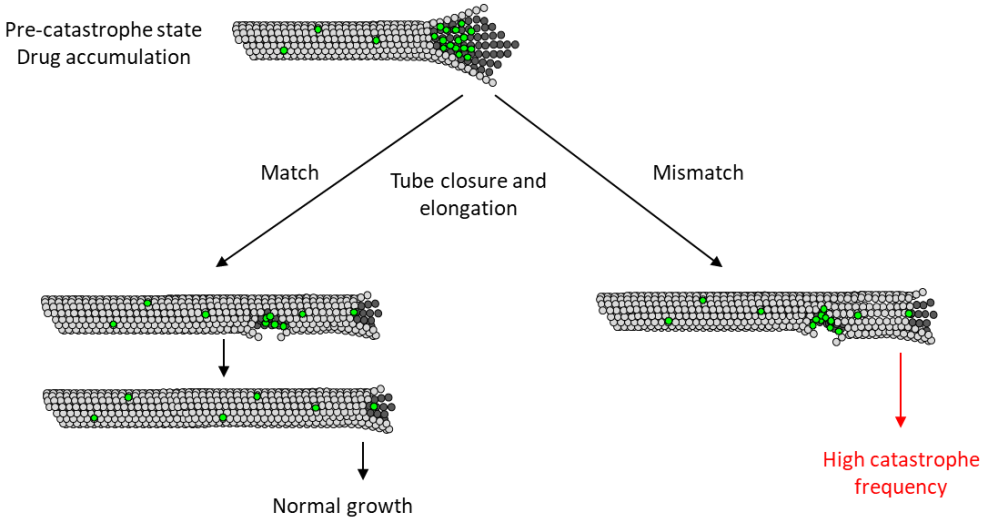

Figure 5: Lattice defects caused by mismatch in protofilament number persist and induce catastrophes.

Our data suggest that structural defects within microtubule lattice can exert effects that can propagate over long distances and affect the dynamic state of the microtubule end (Figure 5) **(Rai et al., 2021, PNAS).** Fundamentally, my work affects thinking on how microtubule dynamics is regulated and helps to explain the enigmatic phenomenon of microtubule aging. An interesting therapeutically relevant implication of these findings is the unexpected cooperativity between microtubule destabilising and stabilising compounds in their ability to inhibit cancer cell growth which open a new window for combination therapy for cancer treatment.

Reference:

1) **Rai A**, Liu T, Glauser S, Katrukha EA, Estévez-Gallego J, Rodríguez-García R, Fang WS, Díaz JF, Steinmetz MO, Altmann KH, Kapitein LC, Moores CA, Akhmanova A. Taxanes convert regions of perturbed microtubule growth into rescue sites.

Nature Materials. 2020 Mar;19(3):355-365. PubMed PMID: 31819210.

Impact factor: 47.656

2) **Rai A,** Liu T, Katrukha EA, Estévez-Gallego J, Paterson I, Díaz JF, Kapitein LC, Moores CA, Akhmanova A. Lattice defects induced by microtubule-stabilizing agents exert a long-range effect on microtubule growth by promoting catastrophes.

Proc Natl Acad Sci U S A. 2021 Dec 21;118(51):e2112261118.

Impact factor: 12.78

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