The Binding Mode of Epothilone A on α,β-Tubulin by Electron Crystallography

James H. Nettles, Huilin Li, Ben Cornett, Joseph M. Krahn, James P. Snyder, Kenneth H. Downing

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Presented By: Rong Hu
Journal Club Members: John Brothers II
Mentor: Dr. Billy W. Day
University of Pittsburgh
Department of Pharmaceutical Sciences
Department of Chemistry

What Do Microtubules Do?

► Intracellular transport

► Motility (cilia and flagella)

► Shape (cytoskeleton)

► Cell division (mitosis)
  - Spindle formation

http://www-vis.lbl.gov/Vignettes/KDowning-Microtubules/
So What Are Microtubules?

► Monomers – α and β-tubulin
  ▪ Nucleotide-binding zone (GTP)

► Dimers – α,β heterodimers
  ▪ Interface includes nucleotide of α-tubulin

► Protofilaments – straight chain of dimers
  ▪ Interface includes nucleotide (GTP → GDP)

► Lateral Contacts – bind protofilaments to form microtubule wall
  ▪ Homogenous contacts (α,α or β,β)
  ▪ M Loop
So What Are Microtubules?

► Left-handed, 3-start helix

► Minus end (stable)
  ▪ Capped with α-tubulin w/ embedded nucleotide (GTP)
  ▪ Lateral contacts strong, independent of nucleotide state

► Plus end (dynamic)
  ▪ Capped with β-tubulin w/ exposed nucleotide (GTP or GDP)
  ▪ Lateral contacts
    ► GTP – strong
    ► GDP – weak

Taxanes: Taxol (paclitaxel)

► Binds to β-tubulin adjacent to the M loop

► Stabilizes lateral contacts of plus end
  ▪ Overrides hydrolysis-induced conformational change in M loop

► Rests in position in β subunit occupied by extra 8 amino acids of S9-S10 loop in α-tubulin
Taxanes vs. Epothilones

- Problems with taxanes
  - Delivery problems
  - Resistance
  - Side effects

- Epothilones (*Sorangium cellulosum*)
  - More water soluble
  - Escape dug resistance encountered by taxanes

- Similar mechanisms
  - Stabilization of microtubules leading to apoptosis

Common Pharmacophore?

- Previous articles concluded there is a common pharmacophore for taxanes and epothilones

- Superposition of epothilone onto taxane

- Verification of toxicity profiles of resistant, mutant cancer cell lines
Methods

► Formation of tubulin/EpoA crystals via zinc stabilization

► Electron crystallography
  ▪ 223 diffraction patterns
  ▪ Tilt ranges 15°-55°
  ▪ More tilted – improved resolution, weaker signal

Methods

► Formation of Ligand Omit Map
  ▪ Start with Taxol/Tubulin complex in PDB
  ▪ Yield model with resolution of 2.89 Å

► NMR and X-ray crystal analysis of EpoA
  ▪ Fit confirmations into electron crystallographic density

► Evaluation of ligand binding
  ▪ QUANTA automated ligand fit
  ▪ Compare with EC density map
No Common Pharmacophore

- EpoA: Extensive hydrogen bonding network
- Taxol: Pairing of aromatic side chains with complementary residues

Explaining Experimental Data

- **Phe**^{270} \rightarrow **Val**^{270}
  - Taxol: 24-fold resistance
  - EpoA: 3-fold resistance
- **Thr**^{274} \rightarrow **Ile**^{274}
  - Taxol: 10x less active
  - EpoA: 40x less active
- **Arg**^{282} \rightarrow **Gln**^{282}
  - Taxol: 7-fold resistance
  - EpoA: 57-fold resistance
- Prevents EpoA binding
  - **Gln**^{292} \rightarrow **Glu**^{292}
  - **Ala**^{231} \rightarrow **Thr**^{231}
Explaining Experimental Data

► C12: alkyl chain extensions are still active
  ▪ C12 substituent directed into hydrophobic basin which accepts long, bulky hydrophobic groups

Explaining Experimental Data

► 14-methyl-epothilone B and D isomers:
  (R)-EpoB and (S)-EpoD active;
  (S)-EpoB and (R)-EpoD inactive
  ▪ (R)-EpoB and (S)-EpoD directs methyl outward, away from protein
  ▪ (S)-EpoB and (R)-EpoD points methyl inward and results in steric clash with CH₂ at C10
Explaining Experimental Data

► C3: hydroxyl (-OH) → cyano (-CN) still active
  ▪ Loss of hydrogen bonding from -OH to Thr274
  ▪ Addition of -CN results in extension with allows it to hydrogen bond with backbone NH of Arg276

Explaining Experimental Data

► Epoxide replaced with double bond still active
  ▪ No direct interaction between oxygen (of epoxide) and protein
  ▪ Both cis and trans forms can participate in key ligand-protein interactions without steric clashes
Explaining Experimental Data

► Thiazole replacements
  ▪ Ortho-pyridine active
    ► Pyridine hydrogen bonds to His$^{227}$
  ▪ Ortho-pyridine, methyl inactive
    ► Hydrogen bond to His$^{227}$ causes ortho methyl to sterically clash with methyl of C16

![ortho-pyridine epothilone](image1)

![pyridine epothilone, both N & Me ortho](image2)

Explaining Experimental Data

► Aziridines replacement of epoxide still active
  ▪ N-substituent steered into hydrophobic pocket

![epothilone A (R = H)](image3)

![epothilone B (R = Me)](image4)

![epothilone aziridines, R = COC$_2$H$_5$, CO$_2$C$_2$H$_5$](image5)
Conclusions

“Instead of a common pharmacophore, tubulin displays a promiscuous binding pocket”

References

► Nettles, James H.; Li, Huilin; Cornett, Ben; Krahn, Joseph M.; Snyder, James P.; Downing, Kenneth H. The Binding Mode of Epothilone A on α,β-Tubulin by Electron Crystallography. Science, 2004; 305: 866-869. (and supporting material at www.sciencemag.org/cgi/content/full/305/5685/866/DC1)


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