velocity and direction dependent on motor crowding and ionic strength but not MT length. By contrast, truncated Cut7 monomers drive only plus end directed MT sliding, indicating that plus end directed strokes are the basal activity of the Cut7 motor head and that directional reversal is an emergent property of interacting head-pairs. We propose a possible mechanism for directional reversal, in which minus ended strokes are inhibited by motor crowding, causing the basal plus end directed activity to dominate.

3930-Pos Board B658

SRC Phosphorylation Regulates the Human Kinesin-5, Eg5, and Disrupts the Binding of Eg5 Inhibitors

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The human kinesin-5 motor, Eg5, is required to establish and maintain the mitotic spindle. We show that Src kinase binds to a unique motif in the microtubule-binding interface of the Eg5 enzymatic head domain and phosphorylates three specific tyrosine residues in endogenous Eg5. These tyrosines are located near the nucleotide pocket and the functionally critical Loop 5 region within the Eg5 head. We have also found that phosphomimetic Eg5 motor proteins have altered motility characteristics relative to wild-type and non-phosphorylatable mutant proteins. Furthermore, cells expressing phosphomimetic Eg5 motors have increased spindle polarity defects. These results implicate Eg5 as a potential direct mitotic target of tyrosine kinases, most likely Src family kinases. Phosphomimetic motors also have greatly reduced affinity for the Eg5 inhibitor S-trityl-L-cysteine (STLC). In cells with high Src activity, including many types of cancers, the same mechanism may provide rapid resistance to therapy with Eg5 inhibitors.

3931-Pos Board B659

Allosteric L5-Directed Inhibitors of Kinesin-5 Can Control Different Biochemical Intermediates

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Kinesin-5 (Eg5) is an essential mitotic motor that couples ATP hydrolysis to different protein-protein association states with microtubules. Human Eg5 is a cancer chemotherapeutic target, having a unique allosteric site covered by loop-5 (L5). This site is capable of binding small-molecule inhibitors with $>$ 100 different chemotypes. The prevailing biochemical model is that all L5-directed drugs inhibit ADP release, but it does not address the 10^7-fold difference in potency or the lack of chemical homology between inhibitor families. An alternative hypothesis is that the inhibitors act on different catalytic intermediates, which gives rise to their disparate potencies. Here we present our linear free energy relationship (LFER) study of Eg5, a method to determine whether an inhibitor can block the catalytic transition-state. Steady-state kinetic parameters for wildtype Eg5 and eight different L5 mutants were determined in the background of three different L5-directed inhibitors and a mock control. The predominant effect of the L5 residue substitution is alteration of substrate binding (K_m), whereas principal outcome of allosteric drug is change in ATP hydrolysis (k_{cat}). Second, our data showed that despite their use of the same binding pocket, one compound can inhibit the transition state and the other two do not. We conclude that the drugs are not synonymous: it is possible for one allosteric modulator to regulate ADP release and another to control transition-state formation. The significance is that this is the first demonstration of allosteric control of more than one catalytic intermediate for any drug target. Furthermore, these results may give insight into the disparity between increased inhibitor potency and success in clinical trials.

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3932-Pos Board B660

Photo-Reversible Inhibition of Mitotic Kinesin Eg5 by Photochromic STLC Analogues Composed of Azobenzene

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The kinesin Eg5, is a microtubule plus-end directed homotetrameric molecular motor that is essential for the formation of a bipolar spindle during eukaryotic cell division. Eg5 exists only in proliferating cells, and is required for mitosis. Therefore, Eg5 is new target of cancer therapy. It is known that some small molecules specifically inhibit Eg5 activity. S-trityl-L-cysteine (STLC) is one of the potent Eg5 specific inhibitors. In this study, we tried to develop photochromic Eg5 inhibitors that control inhibitory activity of Eg5 by ultraviolet (UV) and visible (VIS) light irradiations reversibly. Azobenzene is one of the typical photochromic molecules, which shows cis, and trans isomerization by UV and VIS light irradiations respectively. In this study, we designed and synthesized photochromic inhibitors utilizing azobenzene. As the trityl group of STLC is a key moiety to exhibit inhibitory activity, we linked the trityl group to N-acetyl cysteine or maleic acid via azobenzene. The synthesized two photochromic inhibitors, TAB-MA and TAB-Ac-Cys inhibited the ATPase activity of Eg5 at the different inhibition constants between cis and trans isomers. Trans-isomers of the inhibitors showed more significant inhibition of ATPase activity than cis-isomers. Moreover, Eg5 driven microtubule gliding was photo controlled by TAB-Ac-Cys. Trans-TAB-Ac-Cys decreased the velocity of microtubule gliding more significantly than cis-TAB-Ac-Cys.

3933-Pos Board B661

Photo-Regulation of Kinesin Eg5 ATPase and Motor Activity using Novel Photochromic Inhibitor Composed of Spiropyran and Cysteine

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The mitotic kinesin Eg5 has an important role in establishing the bipolar spindle which is directly involved in the cell division. Recently, several small molecules have been identified as potent specific inhibitor of Eg5. STLC is one of the inhibitor which does not compete with either ATP or microtubules but slows down ADP release. STLC binds to the pocket on Eg5 composed by a2, a3 and loop L5. STLC binding induces the downward swing of L5 to close the inhibitor binding pocket. Therefore, L5 is one of the key region to stabilize Eg5-STLC complex. Previously, we incorporated photochromic molecules into L5 to photo-control inhibitory activity of STLC. Successfully Eg5 mutant D130C modified with spiropyran derivative showed significant photo-reversible resistance to STLC.

In this study, further application of photochromic molecules to regulate Eg5 activity was performed. We synthesized photochromic STLC analogue, IASP-L-Cys composed of spiropyran and L-cystein to regulate Eg5 ATPase and motor activity reversibly upon ultraviolet and visible lights irradiation. IASP-L-Cys exhibited merocyanine - spiro isomerization upon ultraviolet and visible light irradiations. Zwitterionic merocyanine isomer has a ring-opening structure and differs from the other isomer of spiro that is hydrophobic and ring-closing structure. Therefore, it is expected that photo-isomerization of IASP-L-Cys may alter its inhibitory activity for Eg5 significantly. Microtubule dependent ATPase activity of Eg5 was inhibited by IASP-L-Cys in a concentration dependent manner. And the inhibitory activity of IASP-L-Cys for Eg5 was drastically changed correlating to photo-isomerization. Merocyanine isomer showed much higher inhibition constant than that of spiro isomer (approximately 20 μM and 100 μM, respectively). We also examined the effect of phtoisomerization of IASP-L-Cys for the cell mitosis using HeLa cell.

3934-Pos Board B662

Transducer Residues are Thermodynamically Coupled in the Kinesin-5 Motor Domain

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Motor proteins coordinate the activities of nucleotide hydrolysis and polymer binding to move along cellular tracks. In kinesin and myosin motor domains, the sites responsible for these two activities are located on opposite faces of a core beta-sheet. Three beta-strands and a mobile loop transduce information between the active and polymer-binding sites and, thus, are collectively termed the transducer. Herein we demonstrate residues in the core beta-sheet and the L5 loop are thermodynamically coupled. Two methods were employed in this study. Probabilistic methods to analyze evolutionarily correlations between residues in protein families identified coupling between transducer residues. Second, thermodynamic linkage between M115 in loop-5 and L263 in beta-7 in the human kinesin-5 motor domain was established using double mutant cycle analysis for wildtype, two single mutants, and the corresponding double mutant protein. The resulting changes in free energy were calculated from experimentally measured kinetic parameters and determined to be non-additive. While conformational changes of the beta strands and mobile loop have been observed in structural investigations of kinesins and myosins, these experiments establish energetic and evolutionary coupling of these distinct motifs. We conclude that loop-5