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The STARD9/Kif16a Kinesin Associates with Mitotic Microtubules and Regulates Spindle Pole Assembly

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SUMMARY

During cell division, cells form the microtubule-based mitotic spindle, a highly specialized and dynamic structure that mediates proper chromosome transmission to daughter cells. Cancer cells can show perturbed mitotic spindles and an approach in cancer treatment has been to trigger cell killing by targeting microtubule dynamics or spindle assembly. To identify and characterize proteins necessary for spindle assembly, and potential antimitotic targets, we performed a proteomic and genetic analysis of 592 *m*itotic *m*icrotubule *c*opurifying *p*roteins (MMCPs). Screening for regulators that affect both mitosis and apoptosis, we report the identification and characterization of STARD9, a kinesin-3 family member, which localizes to centrosomes and stabilizes the pericentriolar material (PCM). STARD9-depleted cells have fragmented PCM, form multipolar spindles, activate the spindle assembly checkpoint (SAC), arrest in mitosis, and undergo apoptosis. Interestingly, STARD9-depletion synergizes with the chemotherapeutic agent taxol to increase mitotic death, demonstrating that STARD9 is a mitotic kinesin and a potential antimitotic target.

INTRODUCTION

Mitotic spindle assembly is a highly complex and orchestrated event that organizes cell division. Mitosis relies on a multitude of protein complexes, protein-protein interactions, and regulatory mechanisms (Walczak and Heald, 2008). To date, many proteins that associate with microtubules and function in mitotic spindle assembly have been identified and characterized (Loughlin et al., 2008; Manning and Compton, 2008a, 2008b; Walczak and Heald, 2008). Nonmotor proteins, for example, function in microtubule nucleation, crosslinking, and stability, and they can influence the activities of motor proteins (Manning and Compton, 2008b). Motor proteins, in addition to their transport roles, influence microtubule dynamics, kinetochore microtubule attachment, and centrosome separation (Walczak and Heald, 2008).

A strategy in the treatment of cancer has been to inhibit cell division with antimitotic drugs, a set of natural and synthetic small molecules that characteristically arrest cells in mitosis, and induce programmed cell death (Gascoigne and Taylor, 2008; Shi et al., 2008). The spindle is the major target of antimitotics and three major microtubule spindle targets and associated inhibitors have been explored in the clinic: microtubule inhibitors including taxanes and epothilones; inhibitors of the Polo-like kinase Plk1, a regulator of spindle assembly, including BI 2536; and inhibitors of the mitotic kinesin-5, including monastrol and ispinesib (Kapoor et al., 2000; Lansing et al., 2007). Recent clinical trials have questioned the long-term efficacy of current antimitotic drugs. Although taxol remains the most widely-used and efficacious chemotherapeutic agent, it shows dose-limiting toxicities, including neutropenia and severe neuropathies, driving a need to identify alternative antimitotic drug targets that can be targeted and combined with lower doses of taxol to reduce the toxicity associated with high-dose taxol.

To identify proteins involved in mitotic spindle assembly, the linkage to cell death, and thus uncover potential targets for cancer therapeutics, we performed a proteomic analysis to identify *m*itotic *m*icrotubule *c*opurifying *p*roteins (MMCPs) and genetic RNAi screening to test the contribution of these proteins to mitotic progression and induction of apoptosis. We report the results of these screens, which we exemplify by the identification and characterization of STARD9, a mitotic kinesin. STARD9 is

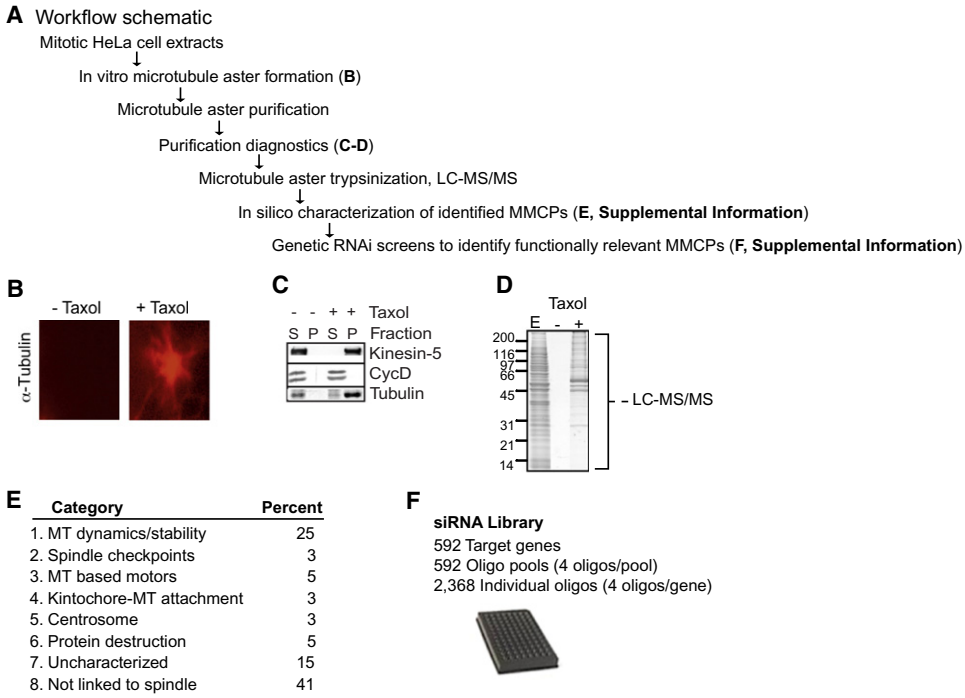

Figure 1. Proteomic and In Silico Analysis of MMCPs

(A) Workflow for purification and identification of MMCPs.

(B) In vitro mitotic aster microtubule polymerization reactions ± taxol, visualized with anti α–tubulin antibodies.

(C) Immunoblot analysis of supernatant (S) and pellet (P) fractions from microtubule polymerization reactions. Blots were probed with anti Kinesin-5, CycD, and α–tubulin antibodies.

(D) Purified microtubule pellets and associated proteins were analyzed by SDS-PAGE and stained with Coomassie blue. E, input extract. Microtubule pellets were digested in solution and proteins identified by LC-MS/MS.

(E) In silico analysis of 592 genes and encoded proteins, for details see text and Supplemental Information.

(F) Generation of a siRNA library targeting 592 genes corresponding to the MMCP set. (A–F) See also Extended Experimental Procedures and Tables S1 and S2.

necessary for PCM cohesion during the establishment of spindle bipolarity. The absence of STARD9 causes the pericentriolar material to fragment and dissociate from the centrioles, along with a failure to congress chromosomes, multipolar spindle formation, mitotic arrest, and apoptotic cell death. Most importantly, depletion of STARD9 synergizes with taxol treatment, making STARD9 a candidate target to extend current cancer therapeutics.

RESULTS

Identification of Mitotic Microtubule Copurifying Proteins

To identify MMCPs that contribute to mitotic spindle formation, we performed a proteomic analysis of microtubule aster copurifying proteins (Figures 1A–1D). Mitotic HeLa cell extracts were induced to undergo in vitro microtubule polymerization in the presence or absence of the microtubule-stabilizing drug, taxol (see Mack and Compton, 2001) (Figure 1B). Polymerized microtubules and associated proteins were purified by sedimentation through a sucrose cushion. The selectivity of the purification was assessed by immunoblotting protein samples from the supernatant (S) and the pelleted microtubule aster (P) fractions for Kinesin-5 and cyclin D (Figure 1C). Kinesin-5 associated with the taxol stabilized microtubule pellet, whereas cyclin D remained in the supernatant (Figure 1C). Neither protein pelleted in the absence of taxol, demonstrating minimal nonspecific pelleting (Figure 1C). Purified microtubule asters were trypsinized in solution and 592 MMCPs were identified by mass spectrometry (LC-MS/MS) (Figure 1D, Table S1, and Supplemental Information available online).

To understand the potential roles of these proteins in cell division, the 592 MMCPs were systematically queried in PubMed and one of eight functional annotations was assigned to each protein based on existing literature (Figure 1E and Table S1). 25% of the proteins had a previously validated role in microtubule dynamics and stability, including TPX2, NuMA, and Astrin. Another 14% were involved in spindle-associated activities, including spindle checkpoints, kinetochore-microtubule attachment, centrosome homeostasis, regulation of mitosis, or were microtubule-based molecular motors including kinesins. Interestingly, over half of the proteins were either uncharacterized or had not been linked to mitotic spindle assembly (Figure 1E and Table S1).

The domain compositions of the 592 MMCPs were analyzed by querying the Unison (http://unison-db.org) (Hart