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STARD9/Kif16a is a novel mitotic kinesin and antimitotic target

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Proper cell division requires the formation of the microtubule-based mitotic spindle, which mediates the dynamic movement and alignment of chromosomes to the metaphase plate and their equal transmission to daughter cells. Kinesins are molecular motors that utilize ATP hydrolysis to perform their functions and are instrumental in spindle assembly and function. Of the over 45 kinesins encoded in the human genome, only two are specifically enriched at the centrioles, Kif24 at the mother centriole and STARD9/Kif16a at the daughter centriole. While Kif24 possesses centriolar microtubule-depolymerizing activity and has been implicated in regulating cilia formation, our recent study implicates STARD9 in maintaining pericentriolar material (PCM) cohesion during early mitosis. However, very little is known about how STARD9 performs its function, including the mechanisms that recruit or retain STARD9 at the centrioles and how it cooperates with centrosome components to regulate PCM stability. Additionally, the signals leading to apoptosis in the absence of STARD9 remain to be explored.

Keywords: STARD9, Kif16a, kinesin, microtubules, centrosomes, spindle assembly, mitosis, cell division, cancer target

Abbreviations: ATP, adenosine triphosphate; STARD9, steroidgenic acute regulatory protein-related lipid transfer (START) domain containing 9; MT, microtubule; MAP, microtubule-associated protein; PCM, pericentriolar material; siRNA, small interfering RNA

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During interphase, mammalian centrosomes play critical roles in organizing the cells microtubule array that functions to define cell shape, polarity and motility.[1] Centrosomes are pivotal for the fidelity of karyokinesis (the division of the nuclear material during cell division), including the organization of the mitotic microtubule spindle, which carries out chromosome congression during prometaphase and chromosome segregation during anaphase of mitosis.[1] At the core of centrosomes are two microtubule-based barrel like structures known as centrioles (**Fig. 1**). Centrioles are composed of nine triplet microtubules that form hollow cylinders.[2] The two centrioles (mother and daughter) can be distinguished from each other, as the mother centriole has added protein-based appendages at its distal end.[2] Centrioles are surrounded by an amorphous protein rich matrix, the pericentriolar material (PCM), which is primarily composed of Pericentrin and CG-NAP (**Fig. 1**).[2] During interphase centrosomes contain a small PCM that expands and matures (recruits additional proteins) at mitotic entry, a process that is necessary to resist the centrosome directed forces that are generated by microtubule associated molecular motors, like dynein and kinesins.[2,3] These forces are not only critical for nuclear envelope breakdown but also for pushing and pulling chromosomes to the metaphase plate. Thus, the PCM has important roles in organizing spindle assembly through microtubule nucleation, elongation, and for resisting forces applied to the centrosome by molecular motors.

Proteomic, genetic, and bioinformatic approaches have been instrumental for defining the parts list of putative centrosome components and regulators of centrosome homeostasis.[4-6] However, little is known about how these components localize to the centrosome, interact with each other, are regulated in a spatial-temporal manner, and coordinate the formation of the mature centrosomes. Recently, we set out to identify novel mitotic cancer targets by characterizing the proteome associated with mitotic microtubule asters, using biochemical purifications and mass spectrometry approaches,

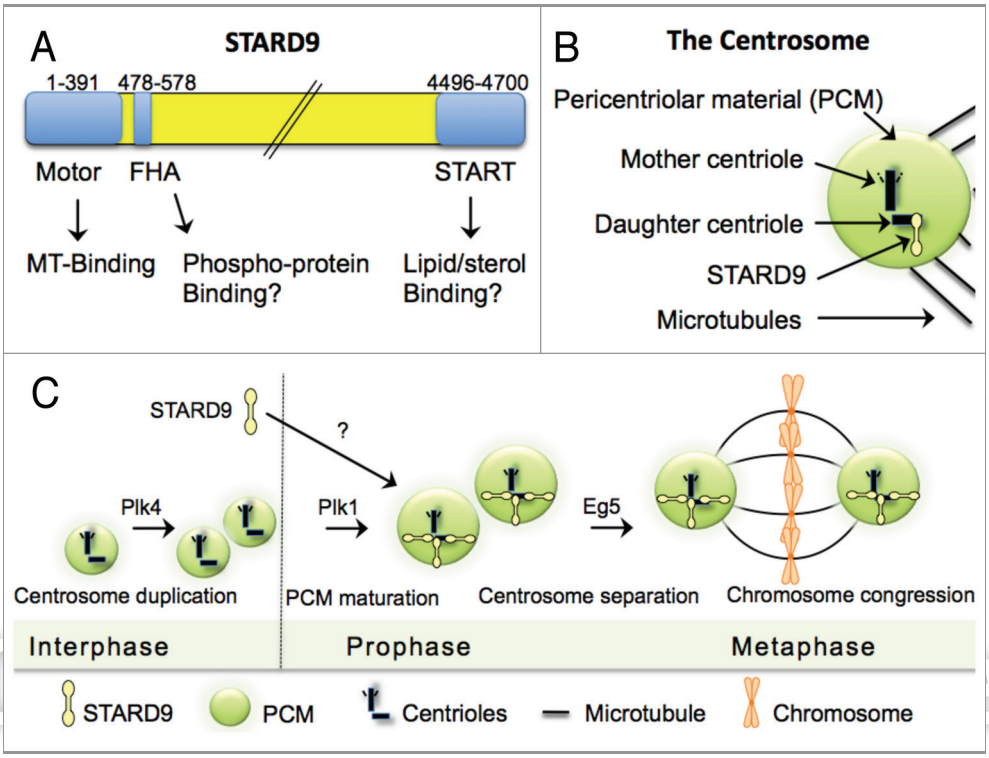

Figure 1. The role of STARD9/Kif16a during cell division. (A) STARD9 is a large ~517 kDa modular protein with an N-terminal kinesin motor domain, an FHA phosphoprotein binding domain, and a C-terminal START lipid/sterol binding domain. (B) Localization of STARD9 to the centrosome. Note that STARD9 is enriched at the daughter centriole. (C) The localization of STARD9 to the daughter centriole occurs during centrosome maturation (after centrosome duplication but before centrosome separation) and is required for PCM cohesion and bipolar spindle assembly.

and by testing their importance for cell division, through genetic small interfering RNA (siRNA) screens for mitotic arrest and induction of apoptosis.[7] Interestingly, we identified STARD9 [steroidgenic acute regulatory protein-related lipid transfer (START) domain containing 9] as a novel mitotic kinesin that localizes to the daughter centriole and is necessary for PCM cohesion during bipolar spindle assembly.[7] STARD9 is a very large (~517 kDa) modular protein with an N-terminal kinesin motor domain, an FHA phosphoprotein binding domain, and a C-terminal START lipid/sterol-binding domain (**Fig. 1A**). Based on sequence homology, STARD9 is a member of the kinesin-3 family, whose members predominantly have roles in the transport of vesicles and organelles.[8-10] Of the over 45 human kinesins, STARD9 and Kif24 are the only two kinesins that are enriched at the mother and daughter centrioles respectively.[7,11,12] Interestingly, they are only conserved in vertebrates, indicating that they may perform specialized functions in higher organisms.[7,11] Currently, we have a limited understanding of what recruits or retains STARD9 or Kif24 to the centrioles. Centrioles contain δ- and ε-tubulin isoforms not usually found in other microtubule based structures.[13-16] In addition, unlike the bulk of polymerized microtubule arrays, centriolar microtubules are highly polyglutamylated and this modification is thought to regulate the association of centrosomal MAPs (microtubule-associated proteins) to the centrioles.[17-21] However, these modifications apply to both centrioles and they are unlikely to be targeting signals for STARD9 or Kif24. Although the microtubule-binding and ATPase activities of the STARD9 motor domain are required for its localization to centrioles, other factors are likely to play a role.[7] Centrosomal components are highly phosphorylated by mitotic kinase families (like Cdk, Plk, Aurora and Nek) in early mitosis.[22] Therefore, phosphorylation of a daughter centriole specific protein and its recognition by the FHA phosphoprotein binding domain of STARD9 could be a plausible targeting mechanism. However, only a handful of proteins have been identified enriched at daughter centrioles, most notably Centrobin.[6,23] These proteins appear to be associated with the daughter centriole throughout the cell cycle.[6,23] Conversely, STARD9 has a dynamic localization and only associates with centrioles from the onset of mitosis to late anaphase, indicating that its localization is regulated in a spatial-temporal manner (**Fig. 1C**).[7] Thus, further experimentation will be necessary to define the mechanisms of STARD9 localization.

The expansion of the PCM during late G2-phase is thought to be critical for the absorption of microtubule and molecular motor-dependent forces during bipolar spindle assembly and the absence of an expanded PCM leads to centrosome fragmentation.[3] The bonds that keep the expanded PCM cohered are largely unknown. Recently, Kizuna was shown to be a "glue" that holds the expanded

PCM together.[24] The depletion of Kizuna led to PCM fragmentation and multipolar spindle formation in human cells.[24] Similarly, depletion of STARD9 leads to PCM fragmentation, multipolar spindles, and mitotic cell death (apoptosis).[7] Like STARD9, Kizuna is only conserved in vertebrates, indicating that higher organisms have more sophisticated mechanisms that reinforce the PCM to deal with centrosome-directed forces.[24] Kizuna binds to Cep72 and this interaction is required for its localization to the PCM, where it associates with CG-NAP, pericentrin and γ-tubulin.[24,25] The mechanism by which STARD9 functions to cohere the expanded PCM has not been determined. Unlike Kizuna that localizes to the PCM, STARD9 localizes to daughter centrioles.[7,11] In addition, its been hypothesized that the mother centriole is predominantly responsible for the nucleation and tethering of microtubules.[26] Thus, how STARD9 contributes to PCM cohesion remains an enigma. It's possible that the localization of STARD9 to centrioles per se is not important for PCM cohesion, but instead its association and/or stabilization of PCM components.

Recently, microtubule associated enzymes with regulatory roles in spindle assembly, like kinases (Plk1, Aurora A and B) and kinesins (Kinesin-5, CENPE) have received considerable attention as potential cancer targets.[27,28] Kinesins are microtubule-dependent molecular motors that possess ATPase activity. In mitosis, kinesins have critical roles in spindle assembly, chromosome congression, and cytokinesis.[29,30] Not surprisingly, the deregulation of most kinesins can lead to the development and progression of many types of cancers.[31] In addition to being required for mitotic progression, kinases and kinesins require ATP hydrolysis to perform their functions. This feature makes them amenable to small molecule inhibition with ATP-competitive inhibitors or allosteric inhibitors. Indeed, clinical trials are underway to test inhibitors targeting these enzymes for their antiproliferation properties.[32,33] However, inhibition of Plk1 or Kinesin-5 leads to a very stable mitotic arrest with a circular arrangement of chromosomes attached to a monopolar spindle.[34,35] In the case of Plk1-inhibition, the PCM is not matured and the chromosomes lack proper microtubule-kinetochore attachments, whereas in Kinesin-5 inhibition the PCM is matured and syntelic kinetochore-microtubule attachments are observed.[34-36] Nonetheless, these cells are able to remain arrested for prolonged periods of time, upwards of 18 h, before they activate an apoptotic response and die. Most importantly these arrests are reversible, if the drug is washed out of these cells they recover and continue to divide. These stable and reversible arrests are a critical problem in the treatment of cancer. With sufficient time, cells are able to slip out of mitosis or wait for drug clearance and can continue to divide. In contrast, STARD9-depleted cells display a fragmented PCM, form multipolar spindles, activate the spindle assembly checkpoint (SAC), arrest in mitosis, and undergo apoptosis.[7] Interestingly, STARD9-depleted cells undergo apoptosis with faster kinetics than Plk1 or Kinesin-5 inhibited cells, indicating that their mitotic arrests differ.[7] Although the triggers that activate the apoptotic pathway in response to antimitotics or STARD9-depletion are poorly defined, it is tempting to speculate that PCM fragmentation, defective microtubule dynamics, or DNA tearing observed in STARD9-depleted cells could be plausible apoptotic triggers. Centrosome expansion/maturation occurs in late G_2 phase and prepares the centrosome for increased microtubule density required in mitotic spindle assembly.[37,38] Stabilization of the expanded pericentriolar material only occurs during mitosis and agents selectively destabilizing this process would be expected to act only on cells undergoing cell division. Because STARD9 is essential, specifically for cohesion of the expanded PCM during mitosis, inhibition of STARD9 is likely to have fewer side effects than drugs that inhibit other essential microtubule-based processes outside of mitosis, like microtubule poisons that have unwarranted neurotoxicities.

Our studies with STARD9-depletions indicate that compromising the integrity of the PCM may be a trigger for apoptosis and that inhibition of STARD9's function may be a viable approach to inhibiting cancer cell division. The PCM fragmentation and spindle assembly defects observed in STARD9-depleted cells are conserved among multiple types of cancer cell lines.[7] However, depletion of STARD9 in a panel of non-small cell lung carcinomas (NSCLC) indicated that only specific subtypes of NSCLC cell lines were sensitive to STARD9-depletion.[7] This raises the possibility that the genetic background of each type of cancer contributes to the cell death response. Genetic variation could modify the timing of mitotic progression, the presence or absence of mitotic checkpoints, or the activation of compensatory mechanisms that could render a cell resistant. The types and subtypes of cancers that are responsive to STARD9-depletion must be defined to fully assess if STARD9-inhibition will be an effective avenue to pursue in inhibiting cancer cell division and to identify markers that will predict anti-STARD9-inhibitor sensitivity. Nonetheless, several lines of evidence indicate that STARD9 should be pursued as a cancer target. STARD9-depleted cells apoptose during mitosis with faster kinetics than current antimitotic treatments and STARD9 possesses an ATP hydrolyzing activity that is essential for its function and amenable to small molecule inhibition.[7] In addition, depletion of STARD9 strongly synergizes with taxol treatment, making STARD9 an appealing candidate target for combined cancer therapies.[7]

The study of STARD9 is in its infancy. Future studies aimed at understanding the mechanisms of STARD9s function and regulation and the factors that render cells sensitive to STARD9-depletion will be invaluable to understanding the mechanisms driving cancer cell division and how inhibition of these mechanisms can be used to treat cancer.

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