A Centrosome Kinase Modulates Antitumor Drug Sensitivity

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In this issue of *Cancer Cell*, Ahmed and coworkers identify SIK2 as a centrosome kinase and show that it controls the localization of the centriole linker protein C-Nap1. Interference with SIK2 function results in loss of normal mitotic spindle function and increased sensitivity to antitumor drugs.

Cancer is a problem largely owing to shifting tumor cell phenotypic heterogeneity due to intrinsic genomic instability (Schvartzman et al., 2010). Tumor progression is a consequence of selection of cancer cells with properties that confer growth advantage, invasion, dispersal along vascular or lymphatic routes, residence at distant metastatic sites, and resistance to therapeutic challenge (Weinberg, 2008). Current cancer treatments, in addition to surgical intervention, include therapies that widely employ antimitotic drugs and/or genotoxic modalities to reduce tumor burden. Because these treatments are often not completely effective, re-emergence from a small number of surviving cells results in tumor recurrence with an overall decrease in treatment sensitivity and poor clinical outcome. For this reason, strategies to discover novel synergistic therapies directed against manifold disparate tumor cell targets to increase treatment efficacy are a high priority.

In order to identify candidate genes required for G2 and G2/M progression as potential new cancer therapeutic targets, Ahmed et al., (2010) employed a reverse genetic screen using pools of siRNA in combination with automated high-throughput analysis of time-lapse live cell imaging and measurement of single-cell DNA content. Their strategy was validated when the screen revealed several known cell-cycle regulators, including Cyclin Dependent Kinase1, Polo Kinase, and Aurora Kinase B, that upon knockdown induced a "ploidy" shift through either delay or arrest of G2/M. Six novel genes were also identified and authenticated as targets that delayed mitotic progression. The authors selected one—the "Salt Inducible Kinase2" (*SIK2*)—for detailed investigation, because while SIK2 was known to function in regulation of cellular metabolism, its potential role in cell-cycle regulation had not been previously documented. Further validation of SIK2 in control of mitosis revealed that loss-of-function resulted in a dramatic increase in the time to complete mitotic progression (two to three times as long as normal). Importantly, many of the *SIK2*-depleted cells also exhibited mitotic catastrophe, cell death, or failure to execute cytokinesis, resulting in polyploidy.

To address the clinical significance of *SIK2* in cancer, the authors used a population-based study of 229 ovarian cancer patients together with gene microarray and quantitative PCR analyses to assess the relationship between clinical outcome and *SIK2* expression levels. They found that elevated expression of *SIK2* significantly correlated with poor outcome following taxane-based chemotherapy for ovarian cancers. Also, a second study comparing paclitaxel versus carboplatin resistant and responsive ovarian cancers confirmed that the *SIK2* expression was higher in paclitaxel-resistant compared to paclitaxel-sensitive cancers, while there was no significant difference for carboplatin. Thus, *SIK2* expression showed a greater influence on outcomes following treatment with mitotic inhibitors than for genotoxic agents.

Next, to elucidate the mechanism underlying the link between *SIK2*, mitotic function, and taxane sensitivity, the authors explored SIK2 localization in cultured cells. Surprisingly, SIK2 showed a distinct juxtanuclear colocalization with γ-tubulin that was not sensitive to nocodazole-induced microtubule depolymerization. Colocalization was seen in 70% of interphase cells and at the spindle poles in virtually all mitotic cells. Additionally, SIK2 localization was mimicked by recombinant GFP-SIK2 and was sensitive to *SIK2* knockdown. Taken together, these observations demonstrate that SIK2 is an intrinsic component of the centrosome, the cell's major microtubule organizing organelle, and they beg the question of just exactly what is SIK2 doing at the "cell center."

In addition to its fundamental role in microtubule organization, the centrosome provides an important structural context for coordinating cell-cycle regulation (Doxsey, 2001; Mikule et al., 2007; Sluder and Hinchcliffe, 2000). Centrosomes consist of three fundamental structural parts, including a core structure consisting of a pair of microtubule-based centrioles that serve as a centrosome organizer; a protein lattice or matrix that surrounds the centrioles called pericentriolar material (PCM), which serves as a framework to anchor microtubule nucleation sites; and finally, γ-tubulin complexes that are responsible for the nucleation of microtubules. Throughout a normal cell cycle, centrioles occur in pairs—cells contain either one or two pair of centrioles depending on the cell-cycle stage, with a pair of centrioles residing at each mitotic spindle pole. At the completion of mitosis and cell division, each daughter cell inherits one centrosome containing a pair of centrioles. During most of this process, centriole pairs, and newly doubled centrosomes remain tethered to one another by linking fibers, consisting in part of the coiled-coil protein, C-Nap1 (Bahe et al., 2005; Fry et al., 1998). Unraveling or dissolution of the linking fibers allows splitting of centriole pairs and separation of newly doubled centrosomes.

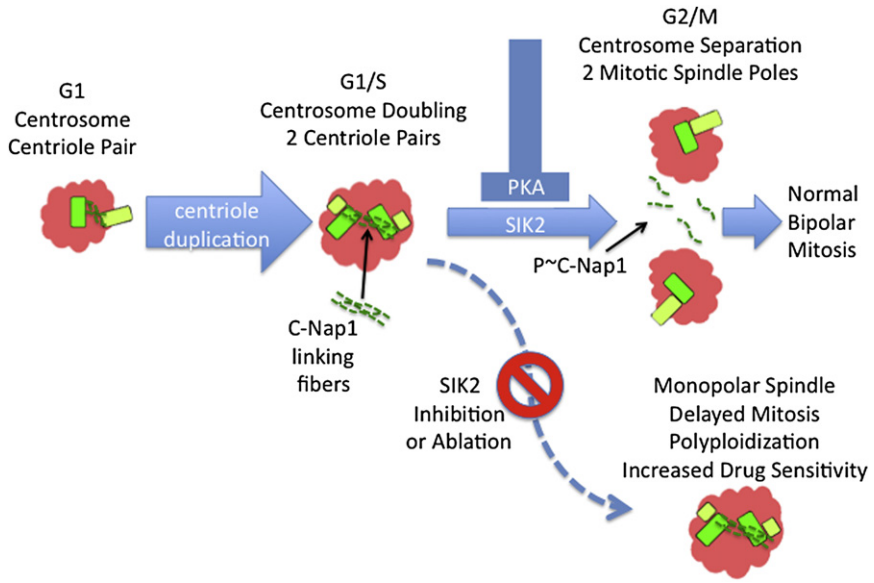

Figure 1. SIK2 Regulates C-Nap1-Mediated Cohesion of Doubled Centrosomes
Centrosomes double once in each cell cycle through the duplication of centrioles (green cylinders) and acquisition of additional pericentriolar material (red cloud). Centriole pairs and doubled centrosomes remain connected through fibers, which are composed in part of C-Nap1. Late in G2 phase of the cell cycle, SIK1 phosphorylates C-Nap1, which solate or detach to allow centrosome separation and bipolar mitotic spindle formation. Inhibition of SIK2 function results in failure of centrosome separation, monopolar spindle formation, and ultimately leads to polyploidization and increased antitumor drug sensitivity.

Remarkably, when Ahmed and coworkers overexpressed SIK2 in cultured cells, they saw precocious centrosome splitting reminiscent of the separation of newly doubled centrosomes in G2/M cells. Furthermore, searching the genome database revealed a putative SIK2 serine phosphorylation consensus in the C-terminal domain of the centrosome linker protein C-Nap1, which they then demonstrated served as a target for in vitro phosphorylation using recombinant SIK2. Coprecipitation and refined colocalization confirmed a close link between SIK2 and C-Nap1. These results and other confirmatory studies highly implicate C-NAP1 as a physiological centrosome target of SIK2.

To further examine the role of SIK2 in mitotic progression and to explain the increased sensitivity of paclitaxel as an antitumor treatment, Ahmed and coworkers re-examined cells in which *SIK2* was ablated using siRNA for centrosome defects. These experiments showed failure of centrosome separation and decreased frequency of bipolar mitotic spindles, which could be rescued by re-expression of recombinant SIK2. By interfering with the centrosome targeting of the known SIK2 inhibitor, protein kinase A (PKA), Ahmed et al. also demonstrated a potential regulatory role for PKA in the timing of centrosome separation in late G2 through control of SIK2.

Taken together, these experiments implicate SIK2 in the control of prometaphase separation of centrosomes giving rise to bipolar mitotic spindle organization through phosphorylation of C-NAP1 (see Figure 1). Furthermore, they suggest that monoploar spindle formation, delayed mitosis, and failure of cytokinesis in SIK2 defective cells can lead to increased sensitization to taxane-based anticancer treatments. The studies by Ahmed and coworkers are exciting because they validate a rationale for discovery of previously unrecognized cell-cycle control processes and they also provide insight into novel avenues for the development of cancer therapeutics that, when used in combination with established treatments, may act synergistically to extend disease free survival and good clinical outcomes.

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