

SIK2 Is a Centrosome Kinase Required for Bipolar Mitotic Spindle Formation that Provides a Potential Target for Therapy in Ovarian Cancer

Ahmed Ashour Ahmed,^{1,4,5,9,*} Zhen Lu,¹ Nicholas B. Jennings,² Dariush Etemadmoghadam,⁷ Luisa Capalbo,⁶ Rodrigo O. Jacamo,³ Nuno Barbosa-Morais,⁸ Xiao-Feng Le,¹ Australian Ovarian Cancer Study Group, Pablo Vivas-Mejia,¹ Gabriel Lopez-Berestein,¹ Geoffrey Grandjean,¹ Geoffrey Bartholomeusz,¹ Warren Liao,¹ Michael Andreeff,³ David Bowtell,⁷ David M. Glover,⁶ Anil K. Sood,² and Robert C. Bast, Jr.^{1,*}

¹Department of Experimental Therapeutics

²Department of Gynecologic Oncology

³Department of Stem Cell Transplantation and Cellular Therapy

M.D. Anderson Cancer Center, University of Texas, Houston, TX 77030, USA

⁴Department of Oncology, University of Cambridge, Hutchison/MRC Research Centre, Hills Road, Cambridge CB2 0XZ, UK

⁵Department of Obstetrics and Gynaecology, University of Cambridge, The Rosie Hospital, Robinson Way, Cambridge CB2 0SW, UK

⁶Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

⁷Cancer Genomics and Genetics Laboratory, Peter MacCallum Cancer Centre, St Andrew's Place, East Melbourne 8006, Victoria, Australia

⁸Computational Biology Group, Department of Oncology, University of Cambridge, Cancer Research UK Cambridge Research Institute,

Li Ka Shing Centre, Cambridge CB2 0RE, UK

⁹Present address: The Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Level 3, Women's Centre, Oxford OX3 9DU, UK

*Correspondence: ahmed.ahmed@obs-gyn.ox.ac.uk (A.A.A.), rbast@mdanderson.org (R.C.B.)

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SUMMARY

Regulators of mitosis have been successfully targeted to enhance response to taxane chemotherapy. Here, we show that the salt inducible kinase 2 (SIK2) localizes at the centrosome, plays a key role in the initiation of mitosis, and regulates the localization of the centrosome linker protein, C-Nap1, through S2392 phosphorylation. Interference with the known SIK2 inhibitor PKA induced SIK2-dependent centrosome splitting in interphase while *SIK2* depletion blocked centrosome separation in mitosis, sensitizing ovarian cancers to paclitaxel in culture and in xenografts. Depletion of *SIK2* also delayed G1/S transition and reduced AKT phosphorylation. Higher expression of *SIK2* significantly correlated with poor survival in patients with high-grade serous ovarian cancers. We believe these data identify SIK2 as a plausible target for therapy in ovarian cancers.

INTRODUCTION

Uncontrolled mitosis is a distinguishing feature of cancer cells that has been effectively exploited for cancer therapy using anti-tubulin drugs such as paclitaxel. The use of primary paclitaxel-based combination chemotherapy increases progression free survival and overall survival in hematological and solid malignancies including ovarian cancer (Martin et al., 2008; McGuire et al.,

1996). Whereas high-grade serous ovarian cancers are known to be highly chemosensitive, a significant proportion of these cancers fail to respond to primary taxane therapy leading to the emergence of resistant disease. There is a pressing need for the discovery of synergistic therapies that may improve ovarian cancer response to taxane-based chemotherapy and overall prognosis. Currently available mechanisms for selective enhancement of taxane response, such as the use of kinesin

Significance

Taxanes stabilize microtubules, inhibit mitosis, induce apoptosis, and produce regression in a fraction of cancers that arise at many sites including the ovary. The anti-tumor activity of taxanes might be increased by concurrently regulating kinases that affect both cell cycle and mitosis. Here we show that SIK2 localizes at the centrosome, phosphorylates the centrosome linker protein C-Nap1 and plays a key role in regulating the onset of mitosis. *SIK2* depletion resulted in profound synergy with paclitaxel in inducing cytotoxicity, whereas higher expression in ovarian cancers correlated with poor prognosis. Independent of its antimitotic role, SIK2 targeting resulted in decreased G1/S transition and low AKT phosphorylation. Thus, SIK2 provides a multimodal therapeutic target in a subset of ovarian cancers.

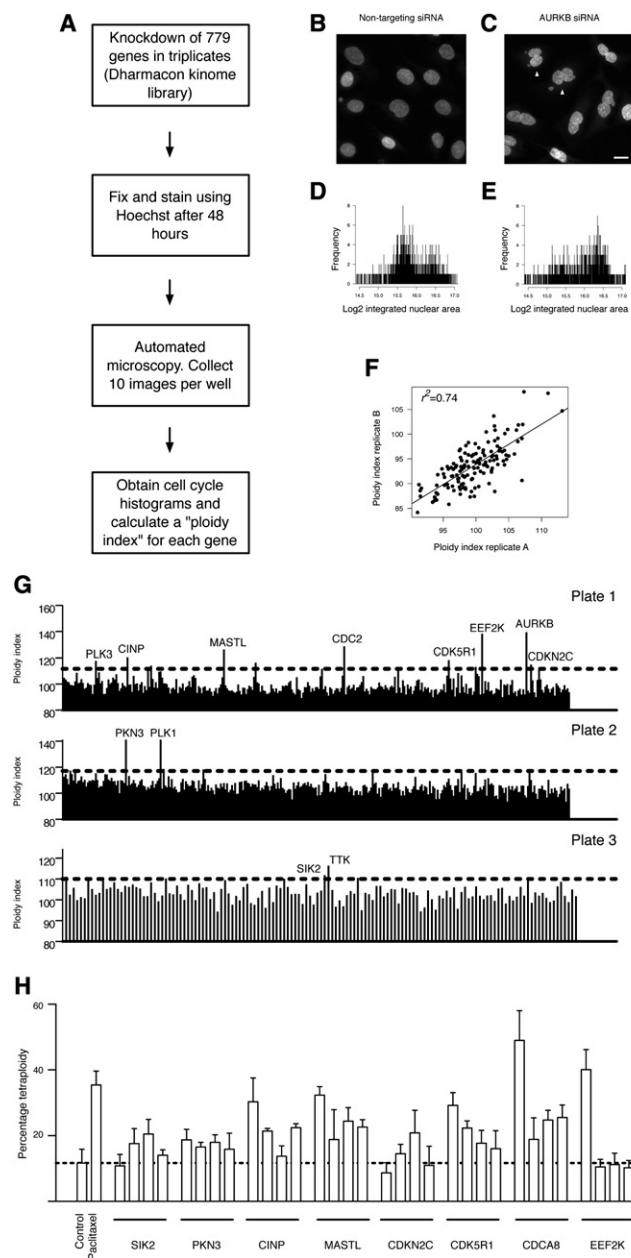


Figure 1. A Kinome siRNA Screen Identifies Regulators of G2/M Progression in Ovarian Cancer

(A) A schematic presentation of the siRNA kinome screen.

(B–H) Each of the 779 genes included in the screen was targeted using pools of four individual siRNAs. These pools were divided into three plates (320 pools for plates 1 and 2 and 139 pools in plate 3). Three replicates were used for each plate, and ten images were obtained per well. Integrated nuclear intensity (INI) values for individual cells, defined as the nuclear area multiplied by its mean pixel intensity, were extracted from 32,400 images, including a total of 1,946,532 cells. The calculation of the percentage shift of the median integrated nuclear intensity value (ploidy index) is explained in (B–E). Images were obtained 48 hr after transfection with (B) nontargeting siRNAs or (C) a pool of siRNAs targeting AURKB. Arrow heads in (C) point to examples of fused nuclei. Scale bar represents 10 μ m. The INI values were used to generate cell-cycle histograms after (D) nontargeting siRNA transfection or (E) AURKB siRNA transfection. Note the shift to the right of the log2 INI values in (E) as a consequence of tetraploidy. The ploidy index (Plnd) was obtained as the

motor inhibitors or the inhibition of mitotic exit, are focused on targeting cancer cells only in mitosis (Huang et al., 2009; Mayer et al., 1999). Although such strategies have shown clinical promise, overall tumor response rates have been limited (Blagden et al., 2008; Gautschi et al., 2008; Lin et al., 2008; Strebhardt and Ullrich, 2006). Novel therapeutic targets that augment taxane effects in inhibiting mitosis while providing taxane- and mitosis-independent mechanisms of cancer cell death are needed to improve clinical chemotherapy response. In this work we conducted an siRNA-based screen to identify modulators of mitotic progression that may influence clinical paclitaxel response.

RESULTS

A High Content siRNA Kinome Screen Identifies Cell-Cycle Regulators of Ovarian Cancer Cells

Recent evidence suggests that delayed mitotic progression or the inhibition of mitotic exit are key predictors of cell death either with or without taxane treatment (Bekier et al., 2009; Huang et al., 2009). To identify regulators of mitotic progression in ovarian cancer cells that may modulate taxane response, we used a three-step approach: (1) we screened 779 pools of siRNAs (Dharmacon kinome library) targeting individual genes to identify potential regulators of the G2 or the M phases (G2/M) of the cell cycle; (2) several hits were selected for further validation using individual siRNAs that made up the pool used in the primary screen; and (3) time-lapse microscopy was used to identify specific regulators of mitotic progression that were then tested for synergistic interactions with paclitaxel.

The primary screen used automated image acquisition and morphometric analysis of fixed cells in 384-well plates (high content analysis, Figure 1A) to measure single-cell DNA content after siRNA treatment. The integrated nuclear intensity values (defined as the nuclear area multiplied by its mean pixel intensity) were calculated as a measure for DNA content and used to generate cell-cycle histograms after the knockdown (KD) of each of the 779 genes included in the screen. The percentage of shift of the median integrated nuclear intensity value after a gene KD compared to that after transfection using nontargeting siRNA was calculated and termed the ploidy index (Figures 1B–1E and Table S1 available online). Genes that on KD induced a percentage shift (ploidy index) that was above the median and 2 standard deviations (SD) of all genes in a plate were identified as positive hits for further validation (Figure 1G and Table S2). There was a high correlation between the ploidy indices across triplicate plates (median correlation coefficient = 0.74) and a mean coefficient of repeatability for all genes tested of 0.1, indicating high data precision in the screen (Figure 1F, Figure S1A,

percentage of the median INI after gene knockdown normalized to the median INI after control transfection. (F) An example of the correlation between the ploidy indices of two replicate plates in the screen. In (G), the cutoff Plnds for each plate and examples of the identified hits are shown. (H) The percentage of the tetraploid cells in relation to the total number of cells after either paclitaxel treatment, as a positive control for tetraploidy, or knockdown of genes using four independent siRNAs. The mean $\pm$ SD of triplicates is presented.

See also Figure S1 and Tables S1–S3.

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