The tumor suppressor CDKN3 controls mitosis

Grzegorz Nalepa, Jill Barnholtz-Sloan, Rikki Enzor, Dilip Dey, Ying He, Jeff R. Gehlhausen, Amalia S. Lehmann, Su-Jung Park, Yanzhu Yang, Xianlin Yang, Shi Chen, Xiaowei Guan, Yanwen Chen, Jamie Renbarger, Feng-Chun Yang, Luis F. Parada, and Wade Clapp

Vol. 201 No. 7, June 24, 2013. Pages 997-1012.

C

0

0

 \mathbf{m}

ш

O

ш

JOURNA

ш

I

The authors noticed an unintended Western blot panel in the original version of Fig. 1. The authors have indicated that this was due to a clerical error during figure revision. A corrected version of Fig. 1 is shown below.

The html and pdf versions of this article have been corrected. The error remains only in the print version.

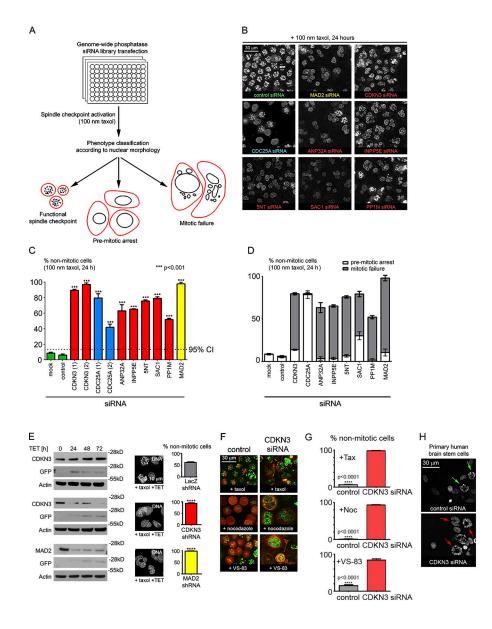


Figure 1. Functional siRNA screen reveals candidate mitotic phosphatases. (A) Screen schematic. 801 siRNAs targeting 267 phosphatases were used in the screen. (B) Nuclear morphology of cells transfected with indicated siRNAs and treated with 100 nm taxol for 24 h 2 d after siRNA transfection. Negative control cells arrest in mitosis when exposed to taxol. Multinucleation resulting from SAC failure occurs in cells transfected with siRNAs targeting CDKN3, ANP32A, INPP5E, 5NT, SAC1, and PP1M as well as MAD2 (positive control). CDC25A knockdown results in premitotic arrest. (C) Quantification of screen results. Broken lines: 95% confidence interval. P-values were calculated using oneway ANOVA. n = 3 counts for each siRNA (a single representative experiment out of two repeats). Error bars represent mean values ± SEM. (D) Subquantification of phenotypes into premitotic arrest versus multinucleation. Knockout of all screen hits except for CDC25A results in SAC failure. Error bars represent mean values of three independent counts (n = 3). (E) Generation of cell lines expressing tetracyclineinducible shRNAs and GFP. Western blots show target knockdown as a function of time in response to shRNA induction. The SAC failure in MAD2 and CDKN3 shRNA cells is indicated by a decreased phospho-H3 fraction in cells exposed to 100 nm taxol for 24 h after 72 h of tetracycline induction (P < 0.0001 for MAD2 and CDKN3 shRNA cells compared with LACZ shRNA cells in one-way ANOVA; n = 10). Error bars show mean values \pm SEM. (F) CDKN3 knockout cells fail to activate the spindle checkpoint in response to three mitotic poisons. (G) CDKN3 knockdown disrupts the SAC response to three spindle poisons. $HeLa^{GFP \cdot H2B/mCherry-\alpha \cdot tubulin}$ cells were transfected with control and CDKN3 siRNAs, and 48 h later treated with inhibitors for 24 h. Cells were fixed and imaged to count nuclear fractions. n = 3 counts per siRNA per condition. P < 0.0001 (t test). Error bars represent mean values ± SEM. (H) CDKN3 is essential for the spindle checkpoint in human primary brain stem cells. The SC-23 cells were challenged with 200 nm taxol 72 h after siRNA transfection. Mitotic arrest occurs in control cells (green arrows), and CDKN3 knockdown led to multinucleation (red arrows).