Synthetic genetic array screen identifies PP2A as a therapeutic target in Mad2-overexpressing tumors

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The spindle checkpoint is essential to ensure proper chromosome segregation and thereby maintain genomic stability. Mitotic arrest deficiency 2 (Mad2), a critical component of the spindle checkpoint, is overexpressed in many cancer cells. Thus, we hypothesized that Mad2 overexpression could specifically make cancer cells susceptible to death by inducing a synthetic dosage lethality defect. Because the spindle checkpoint pathway is highly conserved between yeast and humans, we performed a synthetic genetic array analysis in yeast, which revealed that Mad2 overexpression induced lethality in 13 gene deletions. Among the human homologs of candidate genes, knockdown of *PPP2R1A*, a gene encoding a constant regulatory subunit of protein phosphatase 2, significantly inhibited the growth of Mad2-overexpressing tumor cells. *PPP2R1A* inhibition induced Mad2 phosphorylation and suppressed Mad2 protein levels. Depletion of *PPP2R1A* inhibited colony formation of Mad2-overexpressing HeLa cells but not of unphosphorylated Mad2 mutant-overexpressing cells, suggesting that the lethality induced by PP2A depletion in Mad2-overexpressing cells is dependent on Mad2 phosphorylation. Also, the PP2A inhibitor cantharidin induced Mad2 phosphorylation and inhibited the growth of Mad2-overexpressing cancer cells. Aurora B knockdown inhibited Mad2 phosphorylation in mitosis, resulting in the blocking of *PPP2R1A* inhibition-induced cell death. Taken together, our results strongly suggest that PP2A is a good therapeutic target in Mad2-overexpressing tumors.

cancer therapy target | anticancer drug | aneuploidy | yeast genetics

The spindle checkpoint is a surveillance mechanism that ensures faithful chromosome segregation during mitosis by monitoring the attachment of chromosomes to the spindle microtubules. Several key components of the spindle checkpoint have been identified, such as Mad1, Mad2, Bub1, BubR1, Bub3, Mps1, and Aurora B kinase. The mitotic arrest deficiency 2 (*MAD2*) gene was the first gene of the spindle checkpoint pathway to be characterized (1, 2). Mad2 is a key component of the spindle checkpoint, playing an important role in preventing the loss or gain of chromosomes during cell division (3). Sotillo et al. reported that mice genetically engineered to overexpress Mad2 developed chromosome instability (CIN) and aneuploidy (4). High levels of Mad2 also resulted in the formation of aggressive tumors in multiple organs (5). Recent studies show that overexpression of Mad2 is caused by loss of the tumor suppressor Rb or p53 (6, 7). Mad2 is overexpressed in many cancers (Table S1), such as malignant lymphoma, liver cancer, lung cancer, soft tissue sarcoma, hepatocellular carcinoma, gastric cancer, colorectal carcinoma, and human osteosarcoma (8).

Protein phosphatase 2A (PP2A), an important and ubiquitously expressed serine/threonine phosphatase, dephosphorylates many key cellular molecules such as Akt, p53, and c-Myc. It plays an important role in cellular processes such as cell proliferation, signal transduction, and apoptosis (9). PP2A holoenzymes negatively and positively regulate cell cycle progression by dephosphorylating pocket proteins and multiple cyclin-dependent kinase substrates (10). PP2A consists of a common heteromeric core enzyme, which is composed of a catalytic subunit and a constant regulatory subunit. *PPP2R1A* encodes an α isoform of the constant regulatory

subunit A, and it serves as a scaffolding molecule (11) to coordinate the assembly of the catalytic subunit and a variable regulatory B subunit (12).

On the basis of the hypothesis that a candidate gene's inhibition can induces lethality specifically in Mad2-overexpressing tumors, we investigated the role of *PPP2R1A* in Mad2-overexpressing tumor cells. Inhibition of PP2A killed Mad2-overexpressing cells by increasing Mad2 phosphorylation while suppressing Mad2 protein levels. We propose that the inhibition of PP2A to target Mad2-overexpressing tumors can be a unique strategy for developing potential anticancer therapies.

Results

Synthetic Genetic Array Screen Identifies Target Genes Whose Deletion Causes Synthetic Dosage Lethality in Mad2-Overexpressing Yeast Cells. As Mad2 is overexpressed in many cancer cells (Table S1) (8), this phenotype can be used to specifically kill Mad2-overexpressing tumor cells. We hypothesized that a specific gene whose inhibition causes synthetic dosage lethality (SDL) with Mad2 overexpression can be a target of cancer therapy (13, 14).

Because the spindle checkpoint and cell cycle regulation are highly conserved between yeast and humans (15), we used synthetic genetic array (SGA) technology (16, 17) to screen the haploid deletion *MATa* library (4,541 strains) for candidate genes whose deletion kills Mad2-overexpressing yeast cells. Deletion mutant strains carrying *pGAL-MAD2* were spotted onto dextrose or galactose plates. Because galactose induces the *pGAL1* promoter to overexpress *MAD2*, SDL interactions were determined by comparing the dextrose and galactose plates (Dataset S1).

Significance

The spindle checkpoint is required for proper chromosome segregation. Mitotic arrest deficiency 2 (Mad2), a component of the spindle checkpoint, is overexpressed in many cancer cells. This phenotype can be used to specifically kill Mad2-overexpressing tumor cells. Because the spindle checkpoint pathway is highly conserved between yeast and humans, we performed a screen to identify mutants in which Mad2 overexpression kills yeast cells. The screen revealed that Mad2 overexpression induced lethality in 13 gene deletions. Among the human homologs of the 13 candidate genes, a gene encoding protein phosphatase 2 (PP2A) significantly inhibited the growth of Mad2-overexpressing tumor cells. These results indicate that PP2A can be a specific therapeutic target in Mad2-overexpressing tumors.

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Eighteen candidates showed lethality on galactose plates, and their specificity was confirmed by comparing them with candidate strains carrying the pGAL1 vector only (Fig. 1A). Of the 18 deletion mutant strains, a growth defect was induced by galactose in 5 strains (black) and by Mad2 overexpression in 13 strains (red).

Depletion of PPP2R1A Impairs Growth of Mad2-Overexpressing Cells. The 13 genes that were identified by the SGA screen have putative human homologs (Fig. 1B). Assuming that the SDL between Mad2 overexpression and gene deletion might be conserved between yeast and humans, we studied whether siRNA-mediated knockdown of candidate genes shows lethality with Mad2 overexpression in human cells. Mad2-overexpressing HeLa cell lines were established (Fig. 2A), and GFP-expressing HeLa cells and Mad2-overexpressing HeLa cells were mixed in a 1:1 ratio. Mixed cells were transfected with siRNA targeting the candidate genes, and the ratio of Mad2-overexpressing cells to HeLa-GFP cells was measured by flow cytometry after 72 h. The percentage of Mad2-overexpressing cells was calculated over GFP-negative cells and normalized against that of control luciferase siRNA-treated cells (Fig. 2B and C). Depletion of PPP2R1A significantly reduced the growth of Mad2-overexpressing cells (Fig. 2C).

Depletion of PPP2R1A Inhibits Colony Formation of Mad2-Overexpressing Cells. Potential cell growth inhibition of PPP2R1A on clonogenic survival in Mad2-overexpressing HeLa cells was studied by the

Fig. 1. Synthetic genetic array screen identifies candidate genes whose deletion kills MAD2-overexpressing yeast cells. (A) The yeast haploid deletion MATa library (4,541 strains) screen identified 18 candidate genes. The 18 deletion mutant strains carrying pGAL-MAD2 were spotted onto dextrose (DEX; negative control) or galactose plates (GAL). The deletion mutant strains carrying pGAL only were spotted onto galactose plates as a negative control. (B) The list of yeast candidate genes whose deletion causes synthetic dosage lethality and their human homologs. The E-values were obtained by Blast searches by using BIOBASE's biological databases and the NCBI Blast search.

Fig. 2. Synthetic lethality with Mad2 overexpression in HeLa cells. (A) Cell lysates from HeLa or Mad2-overexpressing HeLa (HeLa-Mad2 O/E) cells were immunoblotted with anti-Mad2 antibody or Hsc70 antibody (a loading control). (B) A schematic figure of the assay. GFP-expressing HeLa cells and Mad2-overexpressing HeLa cells were mixed in a 1:1 ratio. Mixed cells were transfected with the candidate gene's siRNA. After 72 h, the ratio of Mad2-overexpressing cells to HeLa-GFP cells was measured by FACS. (C) The percentages of Mad2-overexpressing cells were calculated over GFP-expressing cells. The relative percentages of Mad2-overexpressing cells were normalized against luciferase siRNA-treated cells.

colony formation assay. Cells were transfected with a negative-control luciferase siRNA or PPP2R1A siRNA (Fig. 3F). After 3 d, 2,000 cells were split into a well of a six-well plate, and colony numbers were counted after 10 d. The viability (%) was normalized; the percentage of surviving colonies of cells transfected with the control luciferase siRNA was arbitrarily set to 100. Mad2-overexpressing cells had fewer colonies than HeLa cells by PPP2R1A siRNA knockdown (Fig. 3A and B).

To confirm the specificity of this synthetic dosage lethal interaction in another cell line, the Mad2-overexpressing human telomerase reverse transcriptase (hTERT) immortalized retinal pigment epithelial cell line hTERT-RPE1 was generated (Fig. 3E). Suppression of colony formation by PPP2R1A siRNA was much higher in Mad2-overexpressing hTERT-RPE1 cells than hTERT-RPE1 cells (Fig. 3C and D).

PP2A Inhibitor Cantharidin Inhibits the Viability of Mad2-Overexpressing Tumor Cells. The PP2A inhibitor cantharidin displays high levels of anticancer activity against a broad range of tumor cell lines (18), but the mechanism of its anticancer activity is not clear. In our study, cantharidin significantly suppressed colony formation in Mad2-overexpressing HeLa cells (Fig. 4A). Mad2 is commonly overexpressed in human osteosarcoma, a type of bone cancer occurring most often in teenagers (19). The osteosarcoma human cell line OS-17 expresses high levels of Mad2 (Fig. 4B). We found that cantharidin strongly inhibited colony formation in OS-17 (Fig. 4C). Weri1 is an Rb-deficient retinoblastoma cell line (20) that has very high expression of Mad2. As Weri1 is made up of floating cells,

Fig. 3. PPP2R1A depletion inhibits colony formation of Mad2-overexpressing cells. (A) HeLa or Mad2-overexpressing HeLa cells (HeLa-Mad2 O/E) were transfected with luciferase siRNA (Luc) or PPP2R1A siRNA. After 3 d, 2,000 cells were split per well of a six-well plate, and cell images were taken after 10 d. (B) The colony numbers were counted in A. (C) hTERT-RPE1 or Mad2-overexpressing hTERT-RPE1 cells were transfected with luciferase siRNA (Luc) or PPP2R1A siRNA. After 3 d, 1,000 cells were split per well of a six-well plate, and cell images were taken after 10 d. (D) The colony numbers were counted in C. (E) hTERT-RPE1 or Mad2-overexpressing hTERT-RPE1 cell lysates were immunoblotted with anti-Mad2 antibody or anti-GAPDH antibody. (F) HeLa cells were transfected with luciferase siRNA (Luc) or PPP2R1A siRNA and immunoblotted with anti-PPP2R1A antibody or anti-GAPDH antibody.

a 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay was performed for cantharidin-treated Weri1 cells. Cantharidin completely suppressed the viability of Weri1 cells at 4 μ M but not of HeLa cells (Fig. 4E). Taken together, our results show that cantharidin inhibits the viability of different types of Mad2-overexpressing cancer cells.

Depletion of PPP2R1A Induces Substantial Mitotic Delay in Mad2-Overexpressing Cells. A recent study showed that depletion of PPP2R1A prolongs mitotic progression from nuclear envelope break down until anaphase (21). To determine whether PPP2R1A depletion induces an arrest of Mad2-overexpressing cells, HeLa cells or Mad2-overexpressing HeLa cells were transfected with luciferase siRNA or PPP2R1A siRNA. After 3 d, cells were fixed with 4% (wt/vol) paraformaldehyde and stained with an antibody against phospho-histone H3 (p-H3). Mitotic cells were recognized by p-H3 labeling and DAPI-labeled condensed chromosomes (Fig. 5A). Transfection of siRNA targeting human PPP2R1A more efficiently induced mitotic delay (23% mitotic index) in Mad2-overexpressing HeLa cells than in control siRNA luciferase (5% mitotic index) (Fig. 5B and Fig. S1). To determine whether the mitotic arrest induced by PP2A inhibition was dependent on the spindle checkpoint, HeLa cells were transfected with luciferase siRNA or BubR1 siRNA, and after 2 d, the cells were treated with nocodazole or cantharidin. Cantharidin induced mitotic arrest in HeLa cells (Fig. 5C), and BubR1 siRNA reduced mitotic delay in both nocodazole- and cantharidin-treated cells. These data indicate that the mitotic arrest caused by PP2A inhibition requires an unperturbed spindle checkpoint.

Next, we investigated how PPP2R1A exerts its effect on Mad2. HeLa cell lysates were immunoprecipitated with Mad2

or PPP2R1A antibodies, and Western blot was performed with Mad2 or PPP2R1A antibodies. Endogenous Mad2 coprecipitated with PPP2R1A in HeLa cells (Fig. 5D). A search on the Eukaryotic Linear Motif resource for Functional Sites in Proteins (<http://elm.eu.org/search/>) revealed that PPP2R1A has a Mad2-binding site. Therefore, it is possible that PPP2R1A directly binds to Mad2.

Knockdown of PPP2R1A Induces Phosphoserine-Mad2. Phosphorylation is the most common mechanism of regulating protein function and transmitting signals throughout the cell. Conformational changes regulate the catalytic activity of the protein. Thus, a protein can be either activated or inactivated by phosphorylation. Because PP2A is a phosphatase and binds to Mad2, we examined whether PPP2R1A depletion affects Mad2 phosphorylation. HeLa cells were transfected with luciferase siRNA or PPP2R1A siRNA. After 3 d, cell lysates were immunoprecipitated with Mad2 antibodies and blotted with anti-phosphoserine (pS) antibody or anti-phosphothreonine (pT) antibody. PPP2R1A knockdown increased both pS-Mad2 and pT-Mad2 but decreased Mad2 expression (Fig. 6A and B). Also, cantharidin significantly increased Mad2 phosphorylation at serines but not threonines (Fig. 6C and D). These results suggest that PP2A dephosphorylates Mad2.

Mad2-4SA Rescues the Lethality Induced by PPP2R1A siRNA. A recent study showed that Mad2 is modified through phosphorylation on multiple serine residues in vivo and that only unphosphorylated Mad2 (Mad2-4SA; serines at 170, 178, 185, and 195 are substituted with alanines) interacts with Mad1 or the APC/C in vivo (22). Another study reported that phosphorylation of Mad2 inhibits its function through differential regulation of its binding to

Fig. 4. Cantharidin inhibits the viability of Mad2-overexpressing tumor cells. (A) Mad2-overexpressing HeLa cells or HeLa cells were split to 800 cells per well of a six-well plate, and cantharidin was added at indicated concentrations the next day. Colony numbers were counted after ~10 d. (B) HeLa cells or OS-17 cell lysates were immunoblotted with anti-Mad2 antibody or anti-Hsc70 antibody. (C) HeLa cells or OS-17 cells were split to 1,000 cells per well of a six-well plate, and cantharidin was added at indicated concentrations the next day. Colony numbers were counted after colony formation. (D) HeLa cells or Weri1 cell lysates were immunoblotted with anti-Mad2 antibody or Hsc70 antibody. (E) Cells were split into a 96-well plate after cantharidin treatment, and cell viability was assessed by the MTT assay.

Fig. 5. The mitotic arrest caused by PP2A inhibition requires an unperturbed spindle checkpoint. (A) HeLa or Mad2-overexpressing HeLa cells (HeLa-Mad2 O/E) were spread on coverslips. The next day, cells were transfected with luciferase siRNA or PPP2R1A siRNA. After 3 d, cells were stained with mouse monoclonal anti-phosphorylated histone H3 (p-H3) antibodies and FITC-conjugated secondary antibodies (green). DNA was stained with DAPI (blue) to visualize prophase, prometaphase, and metaphase cells. Tubulin was stained with mouse anti- β tubulin antibody and Alexa 594-conjugated secondary antibodies (red) to further confirm the cell cycle stages. (B) The number of mitotic cells was counted among more than 600 cells. Mitotic cells were p-H3⁺ and had a characteristic chromosome morphology. (C) HeLa cells were transfected with control luciferase siRNA or BubR1 siRNA. After 48 h, cells were treated with DMSO, nocodazole (250 ng/mL), or cantharidin (2.5 μ M) for 16 h. Cells were stained with mouse p-H3 antibody and FITC-conjugated secondary antibodies (green). The number of mitotic cells was counted by fluorescence microscopy. (D) HeLa cell lysates were immunoprecipitated with anti-Mad2, anti-PPP2R1A, or IgG control antibodies and immunoblotted to analyze endogenous associated proteins.

Mad1 and Cdc20 (23). We hypothesized that the lethality of Mad2-overexpressing cells induced by PP2A suppression is associated with Mad2 phosphorylation. Substitution of four serines (170, 178, 185, and 195) is reported to completely abolish the phosphorylation of Mad2 (24). To test our hypothesis, we generated a Mad2-4SA-overexpressing stable HeLa cell line (Fig. 6F). PPP2R1A siRNA induced exogenous Mad2 phosphorylation, but substitution of serines 170, 178, 185, and 195 completely abolished Mad2 phosphorylation (Fig. 6E). HeLa cells or Mad2-overexpressing HeLa cells or Mad2-4SA were transfected with PPP2R1A siRNA and after 3 d cells were split into 1,000 cells per well of a six-well plate. Colony numbers were counted after 10 d. PPP2R1A knockdown inhibited colony formation in Mad2-overexpressing cells but not Mad2-4SA-overexpressing cells (Fig. 6G). These results indicate that the lethality induced by PPP2R1A siRNA in Mad2-overexpressing cells is dependent on Mad2 phosphorylation.

Aurora B siRNA Decreases Mad2 Phosphorylation in Mitosis and Suppresses the Lethality Induced by PPP2R1A Depletion. PP2A binds and dephosphorylates Aurora B (25, 26). To determine whether Aurora B is involved in the PPP2R1A depletion-induced phenotype, control luciferase siRNA or PPP2R1A siRNA was transfected with or without Aurora B siRNA to HeLa and Mad2-overexpressing HeLa cells, and then a colony formation assay was performed. Aurora B siRNA substantially suppressed the level of Aurora B (Fig. 7C) and rescued PPP2R1A knockdown-induced death of cells (Fig. 7A and B). Next, we examined

whether Mad2 phosphorylation was affected by Aurora B depletion. HeLa cells were transfected with luciferase siRNA or Aurora B siRNA. After 2 d, cells were treated with nocodazole for 8 h, followed by immunoprecipitation of cell lysates with Mad2 antibody and Western blot with phosphoserine and phosphothreonine antibody, respectively. Aurora B depletion reduced Mad2 serine phosphorylation but not threonine phosphorylation during mitosis (Fig. 7D). Taken together, our results showed that Aurora B is required for Mad2 phosphorylation and for the lethality induced by PP2A inhibition in Mad2-overexpressing cells.

Discussion

The increase in the spindle checkpoint protein Mad2 leads to aberrant checkpoint function, as well as aneuploidy and tumorigenesis (4, 6, 8, 27). For faithful segregation of chromosomes at each division, cells must ensure that each pair of sister chromatids is correctly attached to spindle microtubules from opposite poles before the onset of anaphase. Defects in these processes can lead to increased rates of CIN, which is often observed in cancers. Several pieces of evidence support that Mad2 overexpression is sufficient to cause CIN in vitro and in vivo (4). We hypothesized that Mad2 overexpression could specifically make cancer cells susceptible to death. We found that depletion of PPP2R1A increased Mad2 phosphorylation, decreased Mad2 expression, and killed Mad2-overexpressing cells.

Independent of the spindle checkpoint function, Mad2 overexpression might cause CIN by interfering with kinetochore-

Fig. 6. PP2A dephosphorylates Mad2. (A) HeLa cells were transfected with luciferase siRNA or PPP2R1A siRNA. After 3 d, cell lysates were immunoprecipitated with anti-Mad2 antibody, and immunoblotted with anti-phosphoserine antibody or anti-phosphothreonine antibody. (B) Total lysates for A were immunoblotted with anti-Mad2 antibody, anti-GAPDH (control), or anti-PPP2R1A antibody. (C) HeLa cells were treated with cantharidin at indicated concentrations and collected at 8, 24, or 48 h. Cell lysates were immunoprecipitated with anti-Mad2 antibody and immunoblotted with the same antibody as in A. (D) Total lysates for C were immunoblotted with anti-Mad2 antibody or anti-GAPDH. (E) HeLa cells were transfected with Flag-Mad2-expressing plasmid or vector and transfected with indicated siRNA. After 48 h, lysates were immunoprecipitated by ANTI-FLAG M2 Affinity Gel, and the immunoprecipitates were blotted with anti-phosphoserine antibody or anti-flag antibody. (F) HeLa or Mad2-4SA-overexpressing HeLa cell lysates were immunoblotted with anti-Mad2 antibody or anti-GAPDH antibody. (G) HeLa cells, Mad2-overexpressing HeLa (Mad2 O/E) cells, or Mad2-4SA cells were transfected with luciferase siRNA or PPP2R1A siRNA. After 3 d, cells were split to 2,000 cells per well, and colony numbers were counted after ~10 d.

microtubule (k-MT) dynamics (28, 29). Formation of proper k-MT attachments requires a finely tuned balance between stabilizing factors and destabilizing factors (30). Aurora B normally localizes to centromeres during prometaphase and metaphase and regulates kinetochore microtubules by phosphorylating substrates that interact directly with microtubules (31). Inhibition of Aurora B causes increased rates of lagging chromosomes, and reduced centromere localization of Aurora B, in close proximity to its kinetochore substrates, is essential for its function (32).

Kabeche and Compton (32) reported that when Mad2 is overexpressed, Aurora B fails to localize to centromeres, and phosphorylation of a kinetochore substrate is reduced. These results suggest that Mad2 overexpression exerts its influence on k-MT stability by disrupting the centromere localization of Aurora B kinase.

The kinase activity of Aurora B is regulated by its phosphorylation level (25, 26), and PP2A is a well-known negative regulator of Aurora B (26). Thus, depletion of PPP2R1A may enhance Aurora B activity by increasing its phosphorylation (33, 34). Therefore, in Mad2-overexpressing and PP2A-inhibited cells, highly activated

Aurora B might be delocalized from centromeres, leading to cell death. We found that cell death depends on the Aurora B and Mad2 phosphorylation that is regulated by Aurora B, which explains findings from previous studies. However, Aurora B phosphorylated PLK1 but not Mad2 *in vitro* (Fig. S2).

In summary, we propose the following model for the regulation of Mad2 phosphorylation (Fig. 7E): Aurora B regulates Mad2 phosphorylation (a); PP2A binds with Mad2 and dephosphorylates Mad2 (b); and PP2A binds to Aurora B and dephosphorylates Aurora B (c). In addition, our results show that the deletion of PP2A not only regulates Mad2 phosphorylation but also suppresses Mad2 expression levels, which suggests that Mad2 phosphorylation destabilizes the Mad2 protein. Although there was a dramatic reduction in Mad2 levels when PPP2R1A was depleted (Fig. 6A), Mad2 levels were reduced, but not to the same extent when cells were treated with cantharidin (Fig. 6C). The depletion of PPP2R1A led to lack of the protein, which can destabilize the Mad2-PPP2R1A complex. This destabilization may contribute to the reduction of Mad2. As Mad2 overexpression is known to promote aneuploidy and tumorigenesis, decreasing Mad2 levels in Mad2-overexpressing tumors may be beneficial, which could be an alternative model to explain the lethality instead of the model shown in Fig. 7E.

Fig. 7. Mad2 phosphorylation pathways. (A) HeLa cells or Mad2-overexpressing (HeLa Mad2 O/E) cells were transfected with luciferase siRNA or PPP2R1A siRNA with or without Aurora B siRNA. After 2 d, 2,000 cells were split per well of a six-well plate, and colony images were taken after 10 d. (B) The colony numbers in A were counted. (C) HeLa cells were transfected with luciferase siRNA or Aurora B siRNA. After 3 d, cell lysates were immunoblotted with Aurora B antibody or GAPDH antibody. (D) HeLa cells were transfected with luciferase siRNA or Aurora B siRNA. After 2 d, cells were treated with nocodazole, and cell lysates were immunoprecipitated with anti-Mad2 antibody and immunoblotted with a phosphoserine antibody after 8 h. (E) Model of Mad2 phosphorylation.

PPP2R1A knockdown inhibited colony formation in Mad2-overexpressing cells (Fig. 6G). The 4SA mutation rescued this phenotype, but not completely. These results suggest that the lethality induced by PPP2R1A siRNA in Mad2-overexpressing cells is mainly dependent on Mad2 phosphorylation. The results also suggest that Mad2 phosphorylation-independent mechanisms can also contribute to the lethality.

Although most of the 13 yeast genes showed significant homology to the human counterparts (Fig. 1B) (35, 36), the SDL interaction between siRNA and Mad2 overexpression did not seem to be conserved in yeast and human homologs (Fig. 2). This may be because of differences in death mechanisms (e.g., apoptosis) between yeast and humans. However, it needs to be noted that we used specific conditions for the siRNA transfection, as we considered this experiment a “screen”. We may be able to optimize other conditions under which an ideal SDL interaction can be visualized (e.g., by using different types of siRNA sets).

Recently, PPP2R1A was found to be mutated in 7% (3/42) of patients with ovarian clear cell carcinoma. The nature and pattern of the mutations suggest that PPP2R1A functions as an oncogene (37). Cantharidin has potent anticancer activity on many types of

human cancer cells (38, 39) and is used in traditional Chinese and Vietnamese medicine for cancer treatment (40). LB-100 (Lixte), a novel PP2A inhibitor with low toxicity, enhances the effect of cancer chemotherapy by blocking DNA damage-induced defense mechanisms (41). LB-100 has been approved by the Food and Drug Administration for a phase I study. Our study suggests that PP2A is a good target for cancer therapy and also that PP2A inhibitors are more effective at killing Mad2-overexpressing tumor cells.

Materials and Methods

A detailed description of the materials (siRNAs, plasmids, antibodies, and reagents) can be found in *SI Materials and Methods*. SGA analysis is described in detail in the section. The other methodologies (SGA analysis, colony outgrowth assay, MTT assay, immunoprecipitation, Western blot, immunofluorescence microscopy, cell culture, and transfection) used in this work can also be found in *SI Materials and Methods*.

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