Oncoprotein YAP Regulates the Spindle Checkpoint Activation in a Mitotic Phosphorylation-dependent Manner through Up-regulation of BubR1*

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Background: Oncoprotein YAP regulates cell proliferation and tumorigenesis, and a cellular function of YAP in mitosis is largely unknown.

Results: YAP and its mitotic phosphorylation regulate the spindle checkpoint through up-regulation of BubR1.

Conclusion: The findings reveal a novel link between YAP and the spindle checkpoint.

Significance: Our studies indicate a potential mechanism underlying the oncogenic function of YAP through dysregulation of the spindle checkpoint.

The transcriptional co-activator YAP (Yes-associated protein) functions as an oncogene; however, it is largely unclear how YAP exerts its oncogenic role. In this study, we further explored the functional significance of YAP and its mitotic phosphorylation in the spindle checkpoint. We found that the dynamic mitotic phosphorylation of YAP was CDC14-dependent. We also showed that YAP was required for the spindle checkpoint activation induced by spindle poisons. Mitotic phosphorylation of YAP was required for activation of the spindle checkpoint. Furthermore, enhanced expression of active YAP hyperactivated the spindle checkpoint and induced mitotic defects in a mitotic phosphorylation-dependent manner. Mechanistically, we documented that mitotic phosphorylation of YAP controlled transcription of genes associated with the spindle checkpoint. YAP constitutively associated with BubR1 (BUB1-related protein kinase), and knockdown of BubR1 relieved YAP-driven hyperactivation of the spindle checkpoint. Finally, we demonstrated that YAP promoted epithelial cell invasion via both mitotic phosphorylation and BubR1-dependent mechanisms. Together, our results reveal a novel link between YAP and the spindle checkpoint and indicate a potential mechanism underlying the oncogenic function of YAP through dysregulation of the spindle checkpoint.

The Hippo signaling pathway has been elucidated by genetic studies in Drosophila and is highly conserved in mammals (1–5). The protein kinases Mst1/2 (mammalian sterile-20 like, Hippo in Drosophila) and Lats1/2 (large tumor suppressors, Warts in Drosophila) form the core kinase cascade of the Hippo pathway. The WW45 (WW domain-containing protein, Salvador in Drosophila) and MOB1 (Mps one binder, Mats in Drosophila) function as regulatory subunits for Mst1/2 and Lats1/2, respectively. This tumor suppressor pathway controls organ size, cell contact inhibition, stem cell self-renewal, and cancer development through phosphorylating and inactivating the downstream effectors, YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ binding motif). Without inhibition through Hippo signaling (deregulation or inactivation of Hippo core), YAP/TAZ translocate into the nucleus and function as transcriptional co-activators for TEG-domain containing transcription factors and induce expression of target genes that promote cell proliferation/cell cycle progression, survival, and growth and inhibit apoptosis. We, along with others, have demonstrated that YAP promotes oncogenesis by stimulating cell proliferation and is overexpressed or hyperactivated (as shown by nuclear localization) in many types of human malignancies (6–15). Current studies involving YAP focus on determining its overall oncogenic role and targeting it in cancer in various organs/tissues, as well as discerning its role in cross-talk with other signaling pathways. These studies provide important insight into the oncogenic properties of YAP; however, the underlying mechanisms through which YAP exerts its oncogenic function are poorly understood.

Mitosis is a critical step for the integrity of the cell cycle. Aberrations of mitosis often cause chromosome instability and aneuploidy, which are major characteristics of human malignancies (16). Several cellular surveillance mechanisms control the fidelity of cell cycle progression. The spindle checkpoint (also called the mitotic checkpoint) ensures that both chromosome segregation and mitosis are accomplished with great fidelity by arresting the cells in mitosis until all chromosomes are properly aligned at the metaphase plate (17, 18). When activated by mitotic defects such as chromosome misalignment or spindle disorganization, the spindle checkpoint inhibits the activity of the anaphase-promoting complex/cyclosome-cdc20 and arrests cells in pro-metaphase (19). During activation of the
sclerotic checkpoint, most of the spindle checkpoint components, including mitotic arrest deficiency (MAD1 and MAD2), budding uninhibited by benzimidazoles (BUB1 and BUB3), and BubR1 and monopolar spindle 1 (Mps1/TTK) are localized to the kinetochore (19). The spindle checkpoint signaling is highly conserved from yeast to mammals, and dysregulation of the spindle checkpoint members often results in tumorigenesis (20–22).

We have recently discovered that mitotic phosphorylation of YAP by CDK1 is essential for YAP-driven oncogenic transformation, cell migration, and invasion (23). Furthermore, hypermitotic phosphorylation of YAP promoted mitotic defects in immortalized epithelial cells. Despite these observations, however, the biological significance of YAP/mitotic phosphorylation in mitosis is still unknown, and a link between oncogenic YAP and the spindle checkpoint has not been established. Here, we show that YAP controls spindle checkpoint activation through up-regulation of BubR1, a critical component of the spindle checkpoint. Importantly, mitotic phosphorylation is required for both spindle checkpoint activation and BubR1 transcription. Our data identify YAP as a novel regulator for the integrity of spindle checkpoint surveillance and reveal a link between aberrations of mitosis and YAP-driven oncogenesis.

**EXPERIMENTAL PROCEDURES**

**Expression Constructs**—The pcDNA-YAP expression construct has been described (6). Retroviral wild type YAP and YAP mutant constructs have been described (23). Myc-CDC14A/B expression constructs were provided by Dr. Jiri Lukas and have been described (24). The HA-PTEN construct (25) was purchased from Addgene (Cambridge, MA; catalog no. 10750). The Myc-BubR1 construct was made by cloning the BubR1 full-length cDNA (Addgene; catalog no. 23858) into the pcDNA3.1-Myc vector. Point mutations were generated by the QuikChange site-directed PCR mutagenesis kit (Stratagene, La Jolla, CA) and verified by sequencing.

**Cell Culture and Transfection**—HEK293T, HeLa, and MCF-7 cell lines were purchased from ATCC (Manassas, VA). The cell lines were authenticated at ATCC and were used at low (<25) passages. The immortalized pancreatic epithelial cells (human pancreatic nestin-expressing (HPNE))² were provided by Dr. Michel Ouellette (University of Nebraska Medical Center), who originally established the cell line (26), and the HPNE cells were cultured as described (6). HEK293T, MCF-7, and HeLa cell lines were maintained in DMEM from Hyclone (Logan, UT) supplemented with 10% FBS and l-glutamine plus 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen/Life Technologies). Attractene and HiPerFect from Qiagen were used for transient overexpression and siRNA transfections, respectively, following the manufacturer’s instructions. Nocodazole (100 ng/ml for 16–20 h) and Taxol (1 μM for 16 h) (Sigma) were used to arrest cells in mitosis. RO-3306 (CDK1 inhibitor) was from ENZO Life Sciences (Farmingdale, NY). YAP siRNA oligonucleotides were synthesized by GenePharma (Shanghai, China) based on the following target sequences

² The abbreviations used are: HPNE, human pancreatic nestin-expressing; CS, catalytically inactive.
were from Santa Cruz Biotechnology. Mouse monoclonal anti-
Aurora-A antibody was from Sigma. Anti-GST, anti-His, anti-
BUB1, and anti-BubR1 antibodies were purchased from Bethyl
Laboratories (Montgomery, TX). Anti-Aurora-B antibody was
from Abnova. Anti-Thr288 Aurora-A/Thr232 Aurora-B, anti-
Ser127 YAP, and anti-Ser10 H3 were from Cell Signaling Technol-
ogy (Danvers, MA). Immunoprecipitation and Western blotting
assays were done as described (30).

**Cell Migration and Invasion Assays**—*In vitro* analysis of invasion
and migration was assessed using the BioCoat invasion
system (BD Biosciences, San Jose, CA) and Transwell system
(Corning, Corning, NY), respectively, according to the manufac-
turer’s instructions. The invasive and migratory cells were
fixed with 3.7% paraformaldehyde and stained with ProLong®
Gold antifade reagent with DAPI. The relative invasion and
migration rates were calculated as previously described (23, 31).

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**Figure 1.** The cell cycle phosphatase CDC14 associates with and dephosphorylates mitotic phosphorylation of YAP. A, HeLa cells were treated with
nocodazole. Mitotic cells were collected by mechanic shake-off, released into fresh medium, and harvested at the indicated time. YAP was immunoprecipi-
tated, and the immunoprecipitates and the total lysates were analyzed by Western blot analysis with the indicated antibodies. B, HEK293T cells were trans-
fected with various DNAs as indicated. At 36 h post-transfection, cells were further treated with DMSO or Taxol for an additional 16 h, and total cell lysates were
analyzed with the indicated antibodies. C, HEK293T cells were transfected with various DNAs as indicated. At 48 h post-transfection, cells were lysed and
immunoprecipitated with Myc antibody. The immunoprecipitates were probed with the indicated antibodies. Total cell lysates before immunoprecipitation
were used to check the expression of YAP and CDC14A/B. D, HEK293T cells were transfected with various DNAs as indicated. Immunoprecipitation and Western
blot analysis were done as in C. 3A, T119A/S289A/S367A; 3D, T119D/S289D/S367D. E, *in vitro* dephosphorylation assays using CDC14A/B and their CS phosph-
phatases. GST-YAP proteins were first phosphorylated by CDK1-cyclin B complex and used as substrates for dephosphorylation assays. CS, C278S for CDC14A
(C314S for CDC14B); IB, immunoblot; IP, immunoprecipitation; noc, nocodazole.
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Statistical Analysis—Data were analyzed using a two-tailed, unpaired Student’s t test. A p value of <0.05 was considered as indicating statistical significance.

RESULTS

The Phosphatase CDC14B Associates with YAP and Inhibits Its Mitotic Phosphorylation—We recently demonstrated that YAP is dynamically phosphorylated during mitosis (23). Mitotic phosphorylation of YAP quickly diminishes when cells exit mitosis (23) (Fig. 1A), suggesting the presence of a potential phosphatase responsible for the dephosphorylation. The Hippo-mediated YAP Ser\(^{127}\) phosphorylation was not significantly changed during mitotic exit (Fig. 1A). We tested the possibility that CDC14 phosphatase is involved in this process, because the CDC14 phosphatase controls mitotic exit by antagonizing/dephosphorylating the CDK1-phosphorylated substrates in yeast (32) and in mammalian cells (33–40). YAP migration in an SDS-PAGE gel was retarded (because of phosphorylation) following treatment with the spindle poison Taxol (23). Co-transfection of wild type CDC14A or CDC14B, but not their catalytically inactive (CS) mutants, with YAP greatly enhanced the mobility of YAP, suggesting that YAP was not sufficiently phosphorylated when CDC14 was overexpressed (Fig. 1B). Consistent with the mobility shift of YAP, mitotic phosphorylation of YAP at Thr\(^{119}\) and Ser\(^{289}\) was not induced by Taxol treatment in the presence of CDC14 (Fig. 1B). Expression of another cell cycle phosphatase (CDC25A) or its dominant negative mutant or PTEN had no effects on the mobility of YAP (data not shown), confirming the specificity.

The observed CDC14A/B dephosphorylation of YAP prompted us to explore whether YAP forms a complex with CDC14A or CDC14B. Co-immunoprecipitation showed that the CDC14B, but not the catalytically inactive CDC14B (B-CS), associates with YAP, suggesting that the catalytic activity is required for CDC14B to bind with YAP (Fig. 1C). No interaction was detected between YAP and CDC14A or CDC14A-CS (Fig. 1C). To test whether mitotic phosphorylation of YAP Thr\(^{119}\), Ser\(^{289}\), and Ser\(^{367}\) is involved in the interaction of YAP with CDC14B, we mutated all three sites to alanines (3A) or aspartic acid (3D) and performed co-immunoprecipitation. The YAP3A or YAP3D mutant showed similar binding affinity with CDC14B when compared with wild type YAP in transfected cells (Fig. 1D), indicating that CDK1-mediated phosphorylation of YAP is not required for YAP association with CDC14B.

CDC14B Dephosphorylates YAP in Vitro—We further tested whether YAP is a direct substrate for CDC14B phosphatase. Purified wild type and catalytically inactive CDC14B phosphatases were used for in vitro dephosphorylation assays using CDK1-phosphorylated GST-YAP as substrates. Fig. 1E shows that CDK1-mediated phosphorylation of YAP Thr\(^{119}\), Ser\(^{289}\), and Ser\(^{367}\) was greatly reduced by purified wild type CDC14B, and the CS phosphatases failed to dephosphorylate CDK1-phosphorylated YAP (Fig. 1E). Together, these data suggest that YAP was phosphorylated in mitosis and dephosphorylated by CDC14 during mitotic exit.

YAP Is Required for the Spindle Checkpoint Activation—We demonstrated that hyperphosphorylation of YAP was induced by nocodazole or Taxol (23). Because these spindle-damaging agents hyperactivate the spindle checkpoint and cause sustained mitotic arrest (Fig. 2A, top row), we examined the role of YAP in the spindle checkpoint activation. Compared with con-

FIGURE 2. YAP is required for the spindle checkpoint activation induced by spindle poisons. A, representative photos of HeLa cells treated with DMSO or spindle poisons (nocodazole or Taxol) with or without knockdown of YAP. Two independent siRNAs (YAP#1 and YAP #2) are shown. B and C, mitotic index of HeLa cells with or without knockdown of YAP. HeLa cells were transfected with control (lane 1) or siRNAs against YAP (final concentration, 20 nM; lanes 2 and 3). The total protein lysates were subjected to Western blot analysis with the indicated antibodies (B). At 48 h post-transfection, cells were subjected to nocodazole or Taxol treatment for an additional 16 h (C). The mitotic index reflects the percentage of Ser(P)\(^{10}\)H3 positive cells, as revealed by flow cytometry. D and E, mitotic index of MCF-7 cells with or without knockdown of YAP. siRNA transfection, nocodazole/Taxol treatment, and mitotic index analysis were done as in B and C. The data are from three independent experiments and are expressed as means ± S.E. **, p < 0.01; ***, p < 0.001 (t test).
trol cells, YAP knockdown HeLa cells exhibited a significantly impaired mitotic response in the presence of nocodazole or Taxol (Fig. 2, A–C). Similar results were also observed in MCF-7 cells (Fig. 2, D and E). These results indicate that YAP is required for the spindle checkpoint activation induced by spindle poisons.

**Mitotic Phosphorylation of YAP Is Required for the Spindle Checkpoint Activation**—Both HeLa and MCF-7 cells contain wild type p53. We next determined whether YAP controls the spindle checkpoint activation in response to spindle poisons depending on p53 status. Knockdown of p53 had no effect on the mitotic index in nocodazole-treated HeLa and MCF-7 cells (Fig. 3, A and B). We further used the isogenic human colon cancer cell lines HCT116 with (p53+/−) or without p53 knock-out (p53−/−) for this purpose. As shown in Fig. 3C, knockdown of YAP caused a similar spindle checkpoint response in parental and p53 knock-out cells, suggesting that YAP regulates the spindle checkpoint independently of p53.

To investigate the functional significance of CDK1 phosphorylation of YAP in the spindle checkpoint, we performed rescue/complementation experiments in a doxycycline-inducible manner (Fig. 3D). As shown in Fig. 3E, YAP knockdown HeLa cells expressing siRNA-resistant YAP-S127A showed a normal response to nocodazole treatment. However, induction of siRNA-resistant YAP-S127A/3A (a mitotic phosphorylation-deficiency mutant) failed to rescue the phenotype in YAP knockdown cells. These results suggest that mitotic phosphorylation of YAP is critical for activation of the spindle checkpoint induced by spindle poisons. Our data also indicate that the Hippo-mediated phosphorylation of YAP (Ser127) is not required for the spindle checkpoint (Fig. 3E).

**YAP Induces and Associates with BubR1**—The requirement for YAP in the spindle checkpoint activation prompted us to investigate the possible connection between YAP and the spindle checkpoint components. Western blot analysis demonstrated that YAP or YAP-S127A overexpression up-regulated the expression of spindle checkpoint proteins, including BubR1 and MAD2 (Fig. 4, A). The levels of cyclin B, Aurora-B, Mps1/TTK, and BUB1 were not significantly altered (Fig. 4A and data not shown). Accordingly, YAP knockdown reduced the expression of BubR1 and MAD2 (Fig. 4B). Quantitative RT-PCR showed that YAP-S127A overexpression up-regulated BubR1 and MAD2 mRNA levels (Fig. 4, C and D). Interestingly, mutating the mitotic phosphorylation sites to alanines greatly suppressed YAP-S127A-induced BubR1 expression (Fig. 4C).

We further tested whether YAP associates with BubR1 and found that the interaction between YAP and BubR1 was readily detectable at the endogenous level by co-immunoprecipitation (Fig. 4E). The Taxol treatment did not disrupt or increase the interaction between YAP and BubR1, suggesting that the
mitotic phosphorylation of YAP was not directly involved in association with BubR1 (Fig. 4E). Consistent with this observation, the YAP3A or YAP3D (a phosphomimetic) mutant showed similar binding affinity with BubR1 when compared with wild type YAP in transfected cells (Fig. 4F), confirming that CDK1-mediated phosphorylation of YAP was not required for associating with BubR1.

Enhanced Expression of YAP-S127A Increases the Mitotic Index through BubR1—The expression levels of spindle checkpoint proteins need to be tightly controlled (41). Increased BubR1 hyperactivates the spindle checkpoint and causes mitotic delay/arrest. Therefore, we explored whether gain of function of YAP affects the cell cycle and spindle checkpoint. Cell cycle distribution was assessed by FACS analysis of DNA content. FACS analysis revealed that enhanced expression of YAP-S127A resulted in a 2-fold increase of cells in G2/M (Fig. 5A). Interestingly, cells expressing YAP-S127A/3A failed to cause such a cell cycle distribution (Fig. 5A). Immunostaining with phospho-H3 Ser10 (a marker for mitosis) showed that YAP-S127A overexpression caused a significant increase of cells in mitosis (Fig. 5, B and C), and again, this increase was mitotic phosphorylation-dependent (Fig. 5, B and C). Consistent with our observations, the increased mitotic index was also observed in YAP-transgenic mice (42, 43). Considering that BubR1 was up-regulated by YAP-S127A overexpression, the increased mitotic index may be due to sustained activation of the spindle checkpoint. This hypothesis was further supported by observations from nocodazole-treated cells showing that YAP-S127A overexpression augmented the spindle checkpoint activation in immortalized cells (Fig. 5D). These data implicate that activation of YAP hyperactivates the spindle checkpoint and causes mitotic arrest in a mitotic phosphorylation-dependent manner. Interestingly, overexpression of the spindle checkpoint proteins MAD2 or other oncogenes, such as Aurora-A and active Raf (Raf<sup>V600E</sup>), all caused hyperactivation of the spindle checkpoint and subsequent mitotic arrest (20, 44, 45).
We next explored whether up-regulation of BubR1 is required for YAP-S127A-induced mitotic arrest/spindle checkpoint activation. Interestingly, BubR1 knockdown (Fig. 5E) in HPNE-YAP-S127A cells was sufficient to relieve the mitotic arrest (Fig. 5, F and G). Reduction of BubR1 levels had no effects on HPNE-vector or -YAP-S127A/3A cells (Fig. 5F). Together, these data indicate that YAP promotes spindle checkpoint activation via up-regulation of BubR1.

![Flow Cytometry Analysis](image)

**FIGURE 5.** YAP-S127A overexpression increases mitotic index through BubR1. A, flow cytometry analysis for assessing cell cycle distribution in HPNE cells expressing vector, YAP-S127A, and YAP-S127A/3A. B and C, phospho-H3 S10 staining to determine the presence of mitotic cells in cell lines established in A. D, mitotic index of cells expressing vector, YAP-S127A, or YAP-S127A/3A in the presence of nocodazole (50 ng/ml for 10 h). E, Western blot analysis of knockdown of BubR1 in HPNE cells expressing vector, YAP-S127A, and YAP-S127A/3A. F and G, phospho-H3 Ser10 staining to determine the presence of mitotic cells in cell lines assayed in A. Representative photos with p-H3 S10 staining are shown in G. At least 10 fields were scored for p-H3 Ser10-stained cells for each cell line. The data are from three independent experiments and expressed as means ± S.E. *** p < 0.001 (t test). Ctrl, control; 3A, T119A/S289A/S367A; 4A, S127A/3A.

Hyperactive YAP Induces Mitotic Abnormalities in Immortalized Pancreatic Epithelial Cells—Dysregulation of the spindle checkpoint often causes mitotic defects and subsequent aneuploidy (46, 47). We next determined whether YAP is sufficient to trigger mitotic defects. For this purpose, HPNE (immortalized pancreatic epithelial) cells stably expressing vector, YAP, YAP3D, YAP-S127A, and YAP-S127A/3A were established and maintained at similar passages (~25 at the time
of the experiments conducted). As shown in Fig. 6A, immunofluorescence staining showed normal microtubule/spindle formation (revealed by α-tubulin staining) and centrosome (γ-tubulin staining) number during mitosis in vector-expressing cells (Fig. 6, A–C). Consistent with the results in mammary epithelial cells, overexpression of wild type YAP was not sufficient to induce significant mitotic defects in HPNE cells (Fig. 6, B–D). Interestingly, mitotic defects were detected in a significantly higher percentage of cells expressing YAP-S127A or YAP3D. These mitotic abnormalities include disorganization of microtubules and formation of multipolar spindles (Fig. 6, A, B, and E) and centrosome amplification (Fig. 6, A, C, and E). As expected, abnormal chromosomal behaviors (chromosome misalignment, lagging, and mis-segregation) were significantly increased in YAP-S127A- or YAP3D-expressing cells when compared with control cells (Fig. 6, A, D, and E). As a consequence, YAP3D-expressing cells were frequently detected to be aneuploid (Fig. 6, F and G). Importantly, we did not observe significant mitotic defects in YAP-S127A/3A-expressing cells (Fig. 6E). These data suggest that mitotic phosphorylation promotes mitotic abnormalities in immortalized pancreatic epithelial cells, probably through dysregulation of the spindle checkpoint. Together, our studies also indicate that overexpression of active YAP overrides the sustained activation of the spindle checkpoint, allowing cells to proliferate inappropriately with mitotic defects (Figs. 5 and 6) (23).

**Knockdown of BubR1 Partially Suppresses YAP-S127A Oncogenic Activity**—We recently showed that YAP/YAP-S127A promotes migration and invasion in a mitotic phosphorylation-dependent manner in mammary epithelial cells (23). This is also the case in HPNE pancreatic epithelial cells (Fig. 7, A–D). Ectopic expression of YAP and YAP-S127A (to a greater extent)
increased migration (Fig. 7, B and D) and invasion (Fig. 7C) of HPNE cells. Mutating CDK1-mediated phosphorylation sites to alanine (YAP4A) dramatically suppressed YAP-S127A-mediated migration (Fig. 7, B and D) and invasion (Fig. 7C). Accordingly, addition of RO3306 (a CDK1 inhibitor) almost completely suppressed YAP-S127A-driven migration (Fig. 7E) and invasion (Fig. 7F). Using these cell lines and assays, we further tested whether BubR1 is required for YAP activity. Notably, knockdown of BubR1 greatly reduced the invasive activity of YAP-S127A and had no effect on the invasion rate in HPNE-vector cells (Fig. 7, H and J). Surprisingly, reduction of BubR1 did not affect the migratory activity of YAP-S127A-expressing cells (Fig. 7I). Collectively, these data suggest that active YAP (YAP-S127A) promotes its oncogenic activity (e.g.

**FIGURE 7.** BubR1 mediates YAP-S127A-driven invasion in HPNE cells. A, Western blot analysis of established HPNE cells stably expressing vector, YAP, YAP-S127A, and YAP4A (S127A/3A). B–D, cell migration and invasion assays with HPNE cells expressing various YAP constructs. E and F, HPNE cells expressing vector or YAP-S127A were used for migration (E) and invasion (F) assays in the presence or absence of the CDK1 inhibitor RO3306. G, Western blot analysis of BubR1 knockdown in HPNE vector or YAP-S127A cells. H–J, cell invasion (H and J) and migration (I) assays in HPNE-vector and HPNE-S127A cells transfected with control siRNA or siRNA against BubR1. The data are expressed as the means ± S.E. of three independent experiments. ***, p < 0.001 (t test). 3A, T119A/S289A/S367A.
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cell invasion), at least in part, through up-regulating BubR1, a member of the spindle checkpoint.

DISCUSSION

The oncogenic function of YAP has been extensively studied in various types of human malignancies (3, 48); however, the underlying mechanisms through which YAP drives tumorigenesis are less clear. One of the significant findings in this study is that enhanced expression of hyperactive YAP (YAP-S127A and YAP3D) is sufficient to trigger mitotic defects in immortalized nontransformed pancreatic epithelial cells (Fig. 6). Furthermore, our observations also indicate that active YAP induces abnormal mitosis through the dysregulation of the spindle checkpoint (Figs. 2, 3, and 5). Thus, our studies support the theory that YAP plays a critical role in maintaining normal mitosis (i.e. the spindle checkpoint) and that dysregulation of YAP (overexpression of YAP-S127A or YAP3D) leads to mitotic/spindle checkpoint defects, contributing to failure of genome integrity and subsequent oncogenesis. Interestingly, recent reports have also connected other members of the Hippo pathway with mitosis. For example, cells with Lats2 knockdown or mouse embryonic fibroblasts from Lats2-deficient mice show strong mitotic defects, including centrosome amplification, chromosome misalignment, and cytokinesis failure (49). The other core members of the Hippo pathway such as Mst1, Mst2, Mob1/Mats, and WW45 are also involved in mitotic regulation in animals and human cells (50–55). Therefore, we speculate that the Hippo-YAP pathway components control mitotic-related events and that deregulation of their function may result in genome instability and subsequent neoplastic transformation. Although deregulation of these proteins is known to cause mitotic defects, it is largely unclear how these Hippo core components are regulated during mitosis. Furthermore, whether any of these Hippo core members is involved in the spindle checkpoint remains to be determined in future studies.

The spindle checkpoint dysfunction is an important cause of chromosome instability and oncogenic transformation in human cancers (16, 18, 41). As expected, many genes in the spindle checkpoint signaling have been implicated in tumorigenesis. For example, MAD2 or BUB1 overexpression promotes aneuploidy and tumorigenesis in mice (20, 21). MAD2 is a critical mediator of the chromosome instability upon loss of Rb and p53 pathway (22). Moreover, MAD2 and BubR1 overexpression have been found in a wide spectrum of human cancers (56–59). Recent evidence further suggests that the levels of spindle checkpoint genes must be tightly controlled because reduction or increase expression of these genes causes both chromosomal instability/aneuploidy and tumorigenesis (41). Despite the demonstrated role of the spindle checkpoint proteins in tumorigenesis, however, cancer-related mutations are rare in spindle checkpoint genes, like MAD2 or BubR1. Therefore, our data provide evidence that YAP overexpression or hyperactivation constitutes an alternative mechanism for spindle checkpoint dysregulation during oncogenesis.

One of the interesting findings of this study shows an association between YAP and the spindle checkpoint protein BubR1. How these two proteins bind each other and whether this interaction is direct or indirect require future studies. As a spindle checkpoint protein, BubR1 is localized at the kinetochores during metaphase and diminished when cells enter anaphase. It is worthwhile to determine the localization dynamics of YAP during mitosis and whether a fraction of YAP co-localizes with the kinetochores. This study also suggests that BubR1 functions as a novel downstream mediator of YAP in regulating spindle checkpoint activation and cell invasion (Figs. 5 and 7). YAP regulates BubR1 at the transcriptional level (Fig. 4), and BubR1 protein stability was not altered in the presence or absence of YAP (data not shown), suggesting that BubR1 might be a transcriptional target of YAP in mediating spindle checkpoint function. It is currently not known whether BubR1 is required for YAP-driven cellular transformation/oncogenesis. Because YAP is overexpressed or hyperactivated in many types of cancers, future experiments are also needed to further investigate the relationship between the levels of YAP protein or YAP mitotic phosphorylation and spindle checkpoint proteins, including MAD2 and BubR1, in cancer patient samples. Addressing these questions is important because it will not only further define the role and regulatory mechanism of the YAP in mitotic progression but also will be critical for understanding the mechanisms underlying YAP-driven tumorigenesis. Together, we expect these findings to reveal a novel YAP-BubR1 axis that plays critical roles in YAP-driven oncogenesis and to identify this axis as a potential target for cancer therapeutic design.

Acknowledgments—We thank Tom Dao for assistance with confocal microscopy at the imaging core facility at Nebraska Center for Cellular Signaling. We are grateful to Dr. Jiri Lukas (Danish Cancer Society) for the Myc-CDC14A/B constructs. The HA-CDC25A and its dominant negative mutant constructs were originally from Jacob Falck Hansen (Novo Nordisk Biotechnology Fund). The isogenic HCT116 (p53+/− and p53−/−) cell lines were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University). We also thank Dr. Joyce Solheim for critical reading and comments on the manuscript.

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