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Heat Shock Factor 1–Mediated Aneuploidy Requires a Defective Function of p53

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Abstract

Because heat shock factor 1 (HSF1) phosphorylation by Plk1 has been previously reported to be involved in mitotic regulation and p53 function may be involved in this mitotic regulation, we have further examined HSF1 functions in mitotic regulation according to p53 status. Nocodazole-mediated aneuploidy was increased in p53-defective (p53Mut) cells; however, it was not increased in p53 wild-type (p53WT) cells. Phosphorylation of HSF1 at Ser216 was increased in p53Mut cells with increased stability of securin and cyclin B1 in mitosis compared with p53WT cells. The interaction of p53 with Plk1 that was shown in p53WT cells and that induced normal mitotic checkpoint function was not observed in p53Mut cells; instead, the binding of HSF1 with Plk1 and HSF1 phosphorylation at Ser216 were seen in p53Mut cells, which resulted in increased aneuploidy production. Moreover, the interaction affinity of Cdc20 with Mad2 was inhibited in p53Mut cells, whereas the interaction between Cdc20 and HSF1 was increased. From the data, it was suggested that HSF1-mediated aneuploidy was more facilitated in p53-defective cells, indicating the importance of novel mechanisms for p53 function in HSF1-mediated mitotic regulation and genomic instability. [Cancer Res 2009;69(24):9404–12]

Introduction

Spindle damage induces prolonged arrest of cellular mitosis, but the cells eventually enter the G_1 phase despite failed chromosome segregation and/or cytokinesis. Under normal conditions, cells undergo a second, p53-dependent cell cycle arrest and eventually succumb to apoptotic cell death (1–4). However, p53-deficient cells do not undergo a second cell cycle arrest and the cells eventually adapt and become profoundly polyploid or aneuploid in a manner similar to that observed in cells that are deficient for mitotic checkpoint proteins (5–8). Chromosome nondisjunction results in tetraploidization through mitotic failure (9) and p53 has the intrinsic ability to eliminate the production of tetraploid cells that may otherwise proceed to aneuploidy (10). These findings strongly suggest p53 functions in cases of mitotic defect. However, detailed mechanisms on the role of p53 to regulate mitosis have not yet been elucidated.

In mammalian cells, the amount of Plk1 mRNA and protein is regulated in a cell cycle–dependent manner, increasing from a very low level in the G_1 phase to a maximal level during the G_2-M phase (11, 12). The kinase activity of Plk1 is regulated by its phosphorylation, and the kinase activity peaks at M phase (13–15). Plk1 phosphorylates various substrate proteins, including cyclin B1 and Cdc25C (16). In addition to a potential cell cycle regulatory role, Plk1 has been implicated in the genesis and progression of tumors. Some studies have suggested that p53 might be involved in Plk1 depletion–induced apoptosis (17) and Plk1 has been reported to have the ability to phosphorylate p53 and inhibit p53-dependent transcriptional activity and apoptosis (18). Moreover, the binding of Plk1 to p53 could affect the stability of p53 by dephosphorylation at Ser15 (16).

Although less well understood, the activities of heat shock factor 1 (HSF1) extend far beyond classic induction of heat shock proteins (HSP). HSF1 has been shown to regulate up to 3% of the genome in yeast and HSF1 regulation can affect genes that range in function from energy production to signal transduction, small-molecule transport to carbohydrate metabolism, and cytoskeletal organization to vesicular transport (19). The HSF1-mediated stress response and the activity of specific HSPs have both been implicated in the protection of organisms from a broad range of physiologic conditions, including thermal injury, ischemia/reperfusion, and age-related neurodegeneration (20, 21). Little is known about the role of HSF1 in cancer. It has been shown that HSF1 deficiency in mice protects against tumorigenesis as shown both in a classic chemical skin carcinogenesis model and in a genetic model with a clinically relevant oncogenic mutation in p53 (22). In addition, mice lacking HSF1 in cells containing a p53 deletion exhibited a much lower incidence of tumors, displayed a reduced tumor burden, and exhibited increased survival when compared with wild-type littermates (19), suggesting that tumor cells with a p53 deletion or mutation have a much greater dependency on HSF1 function than normal cells. Moreover, HSF1 expression is also elevated in several cancer cell lines (23, 24).

Previously, we reported that HSF1 phosphorylation by Plk1 in mitosis, the binding affinity of HSF1 with Cdc20, and degradation of HSF1 by β-TrCP are important for proper mitotic regulation. Prolonged HSF1 phosphorylation by Plk1 and the binding of HSF1 and Cdc20 affect the metaphase and anaphase transition and produce aneuploidy (25). In this study, we have further characterized the detailed relationship between HSF1 and p53. Aneuploidy production by HSF1 was usually observed in p53 mutant or deficient cells, which was mediated by HSF1 phosphorylation by Plk1. In the case of wild-type p53, Plk1 phosphorylated p53 instead of HSF1, which resulted in more tight binding between Cdc20 and Mad2, and p53 had a normal active function.

Materials and Methods

Plasmids and constructs. Wild-type human HSF1 was cloned into pHACE containing a COOH-terminal HA tag (26). The phosphorylation

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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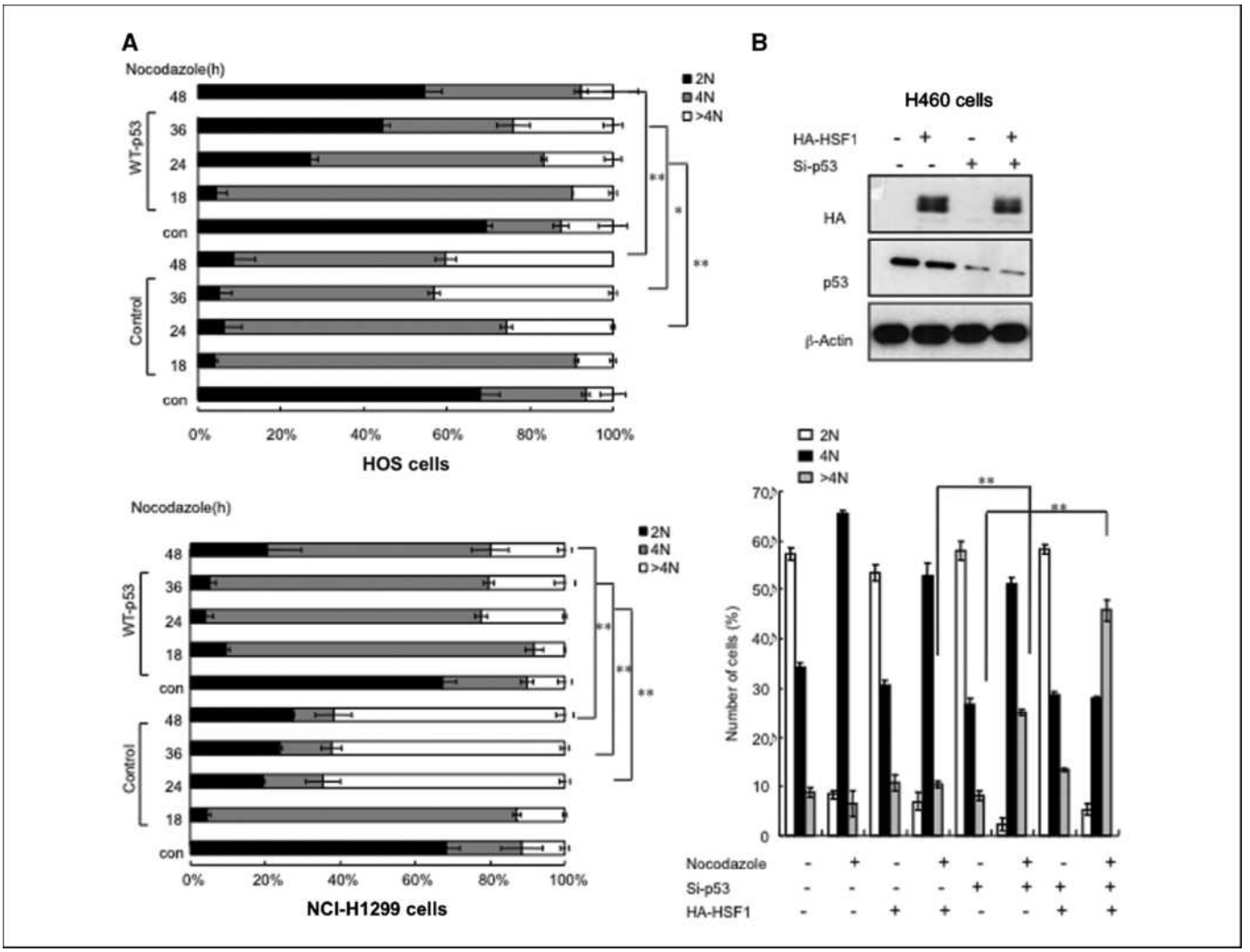

Figure 1. HSF1 mediates aneuploidy production in p53 function–deficient cancer cells. *A,* Hos and NCI-H1299 cells were synchronized by treatment with 100 nmol/L nocodazole and were harvested at the indicated times for flow cytometric analysis. Mitotic and polyploidy cells were distinguished by DNA contents of 4N and >4N, respectively. *Columns,* mean of three independent experiments; *bars,* SD; *, $P < 0.05$; **, $P < 0.01$. *B,* after transfection of wild-type HA-HSF1 into NCI-H460 cells with or without cotransfection of p53 siRNA (Si-p53), cell lysates were analyzed by Western blotting (*top*). Synchronized NCI-H460 cells that were exposed to nocodazole were stained with propidium iodide and were analyzed by flow cytometry (*bottom*). *Columns,* mean of three independent experiments; *bars,* SD; **, $P < 0.01$.

mutant constructs HSF1 (S216N) and HSF1 (S216E) were constructed by using overlap extension primers (25). Plk1 and Plk1 dominant-negative mutants were subcloned into the pCMV5-flag vector. Dr. Bert Vogelstein (Johns Hopkins Oncology Center, Baltimore, MD) kindly provided wild-type human p53.

Cell transfection. Predesigned small interference RNAs (siRNA) for human HSF1 and a negative control were purchased from Dharmacon. Predesigned siRNAs for p53 were purchased from Ambion. Cells were transfected with the siRNAs by the use of Lipofectamine 2000 (Invitrogen) according to the manufacturer's guidelines. siRNA sequences are available in Supplementary data.

Immunoblotting and immunoprecipitation. Immunoblotting and immunoprecipitation were performed essentially as previously described (27).

Cell culture. p53 knockout mouse embryonic fibroblast (MEF; p53+/+ and p53−/− MEF) cells were kindly provided by Dr. H.W. Lee (Yonsei University, Seoul, South Korea). HCT116 (p53WT and p53−/−) cells were kindly provided by Dr. C.W. Lee (Sungkyunkwan University, Suwon, South Korea). The human non–small cell lung cancer cell line NCI-H460 and the human osteosarcoma cell line HOS were also used.

Chemicals and reagents. Nocodazole was purchased from Calbiochem.

Flow cytometry. Flow cytometric analysis was performed using a FACScan flow cytometer (Becton Dickinson).

Kinase assays. Cell lysates were incubated with a Plk1 (Pharmingen) antibody and immunocomplexes were collected on protein A-Sepharose beads and were resuspended in a kinase assay mixture containing [γ-^{32}P] ATP (NEN Life Sciences) and HSF1 protein (Stressgene) as substrates. Proteins were separated on SDS-polyacrylamide gels and the protein bands were detected by autoradiography.

Immunofluorescence analysis. Cells were grown on chamber slides (LabTakII; Nunc) and were imaged under a confocal laser-scanning microscope (Leica Microsystems).

Binding assays *in vitro*. The *in vitro* transcription and translation reactions of Plk1 were performed using a TNT T7 Quick Master Mix kit (Promega) in the presence of [^{35}S]methionine, according to the manufacturer's protocol.

Statistical analysis. Statistical significance was determined using the Student's *t* test for comparison between the means. Mean values were given as the mean ± SD. A null hypothesis was rejected whenever a *P* value of 0.05 or less was determined.

Results

HSF1-mediated aneuploidy in cancer cells with a p53-deficient function. Nocodazole treatment significantly induced

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a large amount of aneuploidy in HOS cells (human osteosarcoma cells that contain a mutant p53) and NCI-H1299 cells (a human non–small cell lung cancer cell lines with a p53 deletion) that have high expression of HSF1 (Fig. 1*A*). Because HSF1-mediated aneuploidy was not observed in p53 wild-type cells such as NCI-H460 cells (data not shown), knock down of p53 with the use of siRNA was performed, which allowed HSF1-mediated aneuploidy after adaptation from nocodazole block (Fig. 1*B*). These results suggest

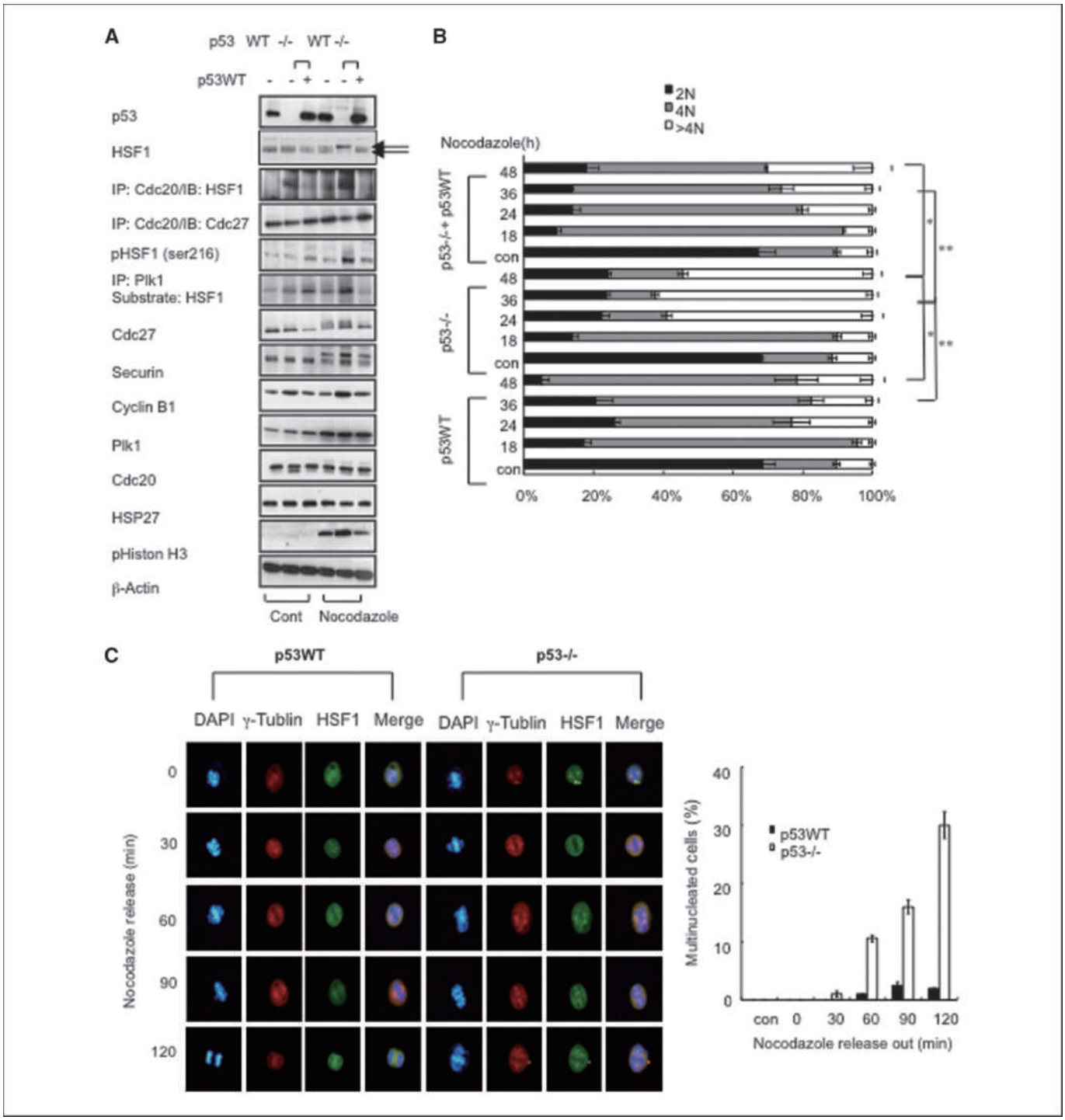

Figure 2. Inhibition of HSF1 phosphorylation and HSF1-Cdc20 binding by wild-type p53. *A,* p53WT and p53–/– HCT116 cells that were transiently transfected with control or wild-type p53 plasmid were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h). The mitotic cells were harvested and lysates were generated for immunoprecipitation, immunoblotting, and kinase assay. *B,* cells were transiently transfected with control or p53WT vector and harvested at the indicated times after nocodazole treatment. The percentage of the DNA content of cells was determined by flow cytometry. *Columns,* mean of three independent experiments; *bars,* SD; *, $P < 0.05$; **, $P < 0.01$. *C,* p53WT and p53–/– HCT116 cells were released from nocodazole (100 nmol/L) arrest after 16 h and were stained for HSF1 (*green*), γ-tubulin (*red*) and 4′,6-diamidino-2-phenylindole (*DAPI*). Cells were analyzed using immunofluorescence microscopy at different mitotic stages. The histograms summarize the results of three independent experiments (with at least 400 cells counted in each experiment in each column; *right,* mean ± SD).

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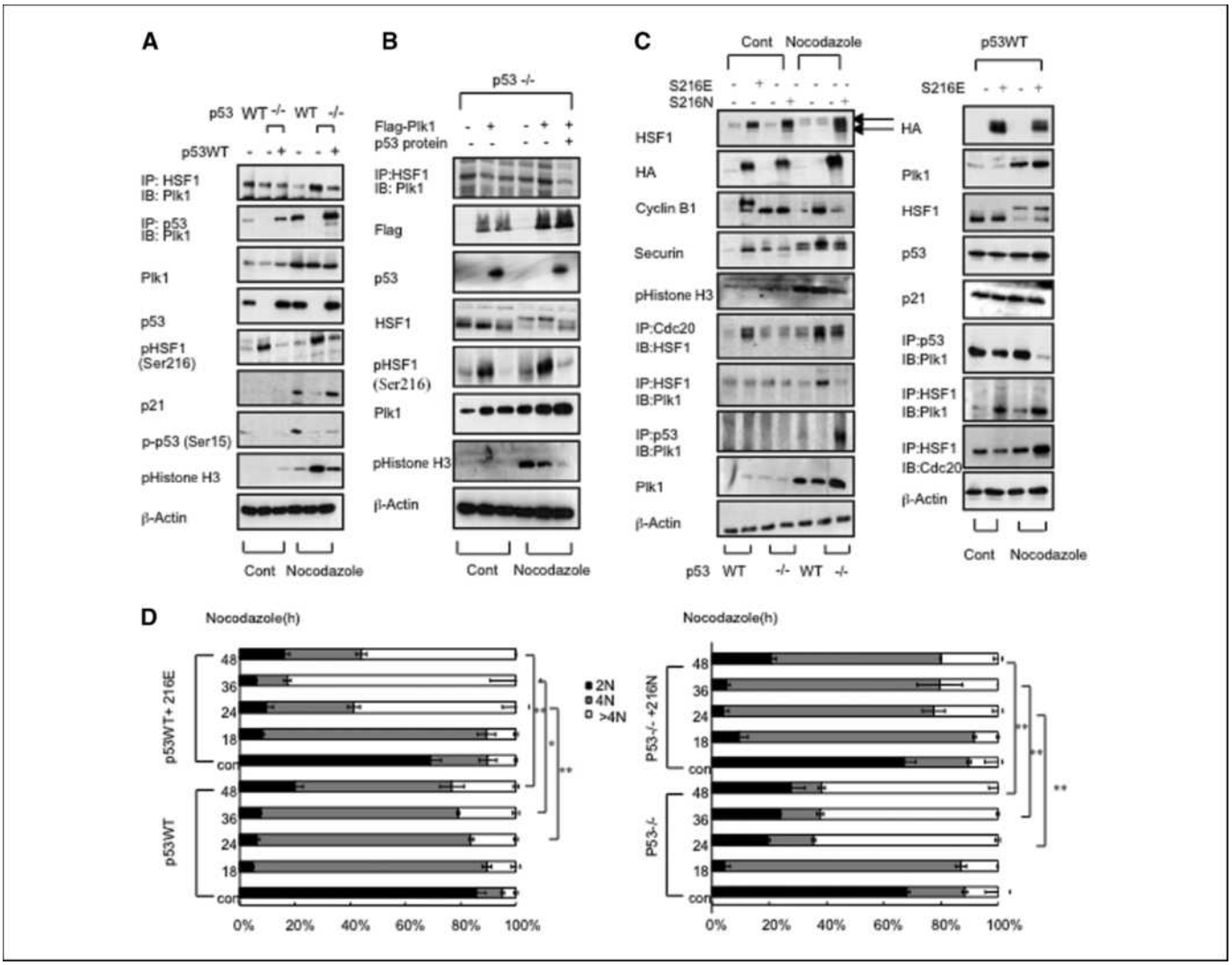

Figure 3. Inhibition of Plk1-mediated HSF1 phosphorylation by wild-type p53. *A,* cells transiently transfected with control or p53WT were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h). The mitotic cells were harvested and lysates were generated for immunoprecipitation and immunoblotting. *B,* p53–/– HCT116 cells were transfected with Flag-Plk1 and were treated with nocodazole (100 nmol/L nocodazole, 16 h). *In vitro* translated ^{35}S-labeled HA-HSF1 was incubated with Plk1 immunoprecipitates and was subjected to an *in vitro* binding assay. *C,* p53WT and p53–/– HCT116 cells were transiently transfected with HA–HSF1 S216E and S216N. Cells were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h). The mitotic cells were harvested and lysates were generated for immunoprecipitation and immunoblotting. *D,* the percentage of the DNA content of cells after nocodazole treatment was determined by flow cytometry. *Columns,* mean of three independent experiments; *bars,* SD; *, $P < 0.05$; **, $P < 0.01$.

that a functional p53 may overcome HSF1-mediated aneuploidy production. We here excluded the possibility that HSF1 directly regulates p53, because we could not detect the direct binding affinity between p53 and HSF1 (Supplementary Fig. S1).

Intact p53 function inhibits HSF1 phosphorylation at Ser216 and inhibits HSF1-Cdc20 binding. HSF1 is phosphorylated by Plk1 at Ser216 in early mitosis with binding affinity between phosphorylated HSF1 with Cdc20 in the mitotic transition (23). It was examined if these phenomena were related to the p53 status. p53 wild-type (p53WT) HCT116 colon carcinoma cells showed normal cell cycle progression after nocodazole block and release. Phosphorylation of Cdc27 and histone H3 was normal, as was the degradation of securin and cyclin B1. However, for p53-negative (p53–/–) HCT116 cells, phosphorylation of Cdc27 and histone H3, indicative markers of mitotic period (28), were inhibited and the stability of securin and cyclin B1 increased. Moreover, prolonged phosphorylation of HSF1 at Ser216 was also seen in p53–/– cells. When a p53WT plasmid was additionally transfected into the p53–/– cells, these effects were restored to the level as seen in p53WT cells. HSF1 phosphorylation at Ser216 in mitosis as well as increased binding activity between HSF1 and Cdc20 only occurred in p53–/– cells after nocodazole block and release. Inhibition of Cdc27 phosphorylation and increased stability of cyclin B1 and securin were also observed in p53–/– cells as compared with p53WT cells, indicating mitotic arrest (Fig. 2*A*; Supplementary Fig. S2*A*). Similar effects were seen in both p53 wild-type (p53+/+) and p53 deletion (p53–/–) MEFs (Supplementary Fig. S2*B*). Aneuploidy production after adaptation to nocodazole showed that p53–/– cells exhibited a higher level of aneuploidy than p53WT cells; however, with an additional transfection of p53WT plasmid to p53–/– cells, these effects were restored to the level of p53WT cells (Fig. 2*B*). In addition, when we examined aneuploidy production after nocodazole adaptation after transfection of Si-HSF1 into both p53–/– and p53WT cells, HSF1-mediated aneuploidy

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production was more abundant in p53−/− cells (Supplementary Fig. S3). The use of confocal microscopy also showed that p53WT cells apparently underwent normal mitotic progression after release from nocodazole block. However, p53−/− cells showed a defective metaphase-anaphase transition and finally produced multinuclear cells. When we examined the subcellular localization of endogenous HSF1 in p53WT and p53−/− cells from prometaphase through anaphase, HSF1 was seen with a prominent centrosome localization, as judged by costaining for γ-tubulin. The distribution of HSF1 was clearly concentrated to the spindle pole in metaphase regardless of the p53 status. Cells with p53WT apparently underwent normal mitosis after release from nocodazole block. In p53−/− cells, massive chromosome missegregation without the organization of the chromosomes in a metaphase plate was observed. When we detected multinucleated cells in this situation, significantly increased multinucleated cells in p53−/− cells were observed (Fig. 2*C*). Immunofluorescence analysis of p53−/− and p53+/+ MEF cells also indicated that defective function of p53 is involved in HSF1-mediated aneuploidy production (Supplementary Fig. S4).

Wild-type p53 function inhibits Plk1-mediated HSF1 phosphorylation at Ser216. It has been reported previously that p53 interacts with Plk1 (16) and HSF1-mediated aneuploidy production was only observed in functionally p53-defective cells (Fig. 1). We examined the relationship between these molecules and the dependency on the p53 status. Plk1 directly bound to p53 in nocodazole-treated p53WT mitotic cells. For p53−/− cells, Plk1 dominantly interacted with HSF1 instead of p53. HSF1 phosphorylation at Ser216 occurred only in p53−/− cells (Fig. 3*A*). When we directly added p53 protein to p53−/− cell lysates, increased HSF1-Plk1 binding activity and HSF1 phosphorylation at Ser216 in mitotic cells were inhibited. With additional treatment of p53 protein, in a large number of mitotic cells after nocodazole treatment in p53−/− cells, phosphohistone H3 expression was inhibited (Fig. 3*B*). These results indicate that phosphorylation of HSF1 by Plk1 and proper segregation of mitotic cells was dependent on the p53 status. To elucidate whether phosphorylation at Ser216 by Plk1 is critical for HSF1-mediated aneuploidy production in p53−/− cells, mutant forms of HSF1 at Ser216 were prepared. A mutant of HSF1 at Ser216 (S216E) that mimicked phosphorylation of Ser216 showed binding activity with Plk1 in p53−/− mitotic cells as well as increased binding activity of HSF1 and Cdc20, even in nocodazole-untreated control cells. These effects were not observed with a phosphorylation-defective mutant (S216N). Moreover, when S216E was transfected into p53WT cells, the p53-Plk1 interaction was inhibited, whereas interactions of HSF1-Plk1 and HSF1-Cdc20 were increased in nocodazole-treated mitotic cells (Fig. 3*C*). Increased aneuploidy by nocodazole treatment in p53−/− cells was significantly inhibited by transfection of S216N. For p53WT cells, transfection with S216E increased aneuploidy production (Fig. 3*D*). From these findings, an intact function of p53 is involved in blocking aneuploidy production by inhibition of the Plk1-HSF1 interaction and HSF1 phosphorylation at Ser216.

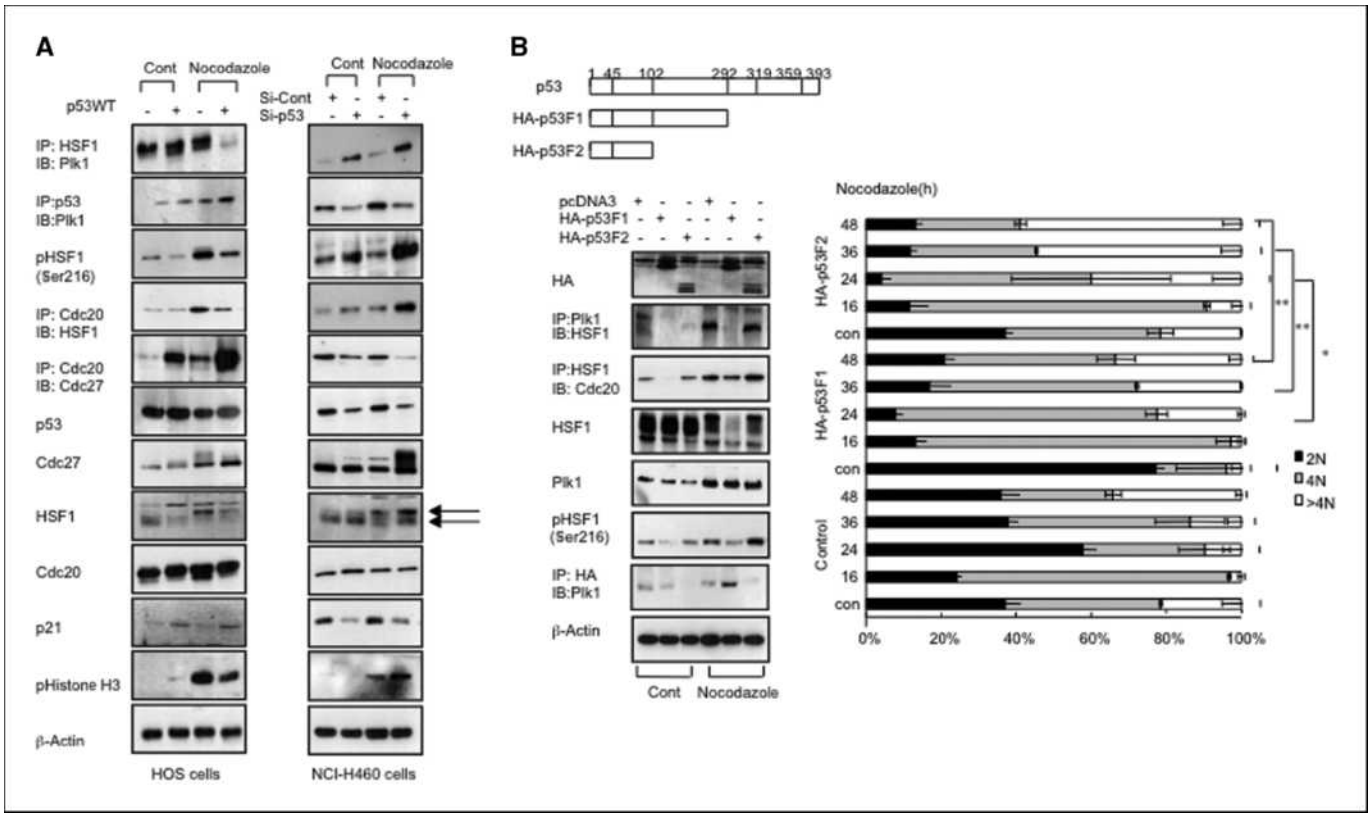

Figure 4. Inhibition of HSF1-mediated aneuploidy by p53-Plk1 binding. *A,* Hos cells transiently transfected with control or p53WT were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h; *left*). NCI-H460 cells transiently transfected with control or Si-p53 were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h; *right*). The mitotic cells were harvested and lysates were generated for immunoprecipitation and immunoblotting. *B,* Hos cells were treated with vectors expressing for HA-F1 or HA-F2. Cells were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h) and lysates were generated for immunoprecipitation and immunoblotting (*left*). The percentage of the DNA content of cells after nocodazole treatment was determined by flow cytometry (*right*). *Columns,* mean of three independent experiments; *bars,* SD; *, $P < 0.05$; **, $P < 0.01$.

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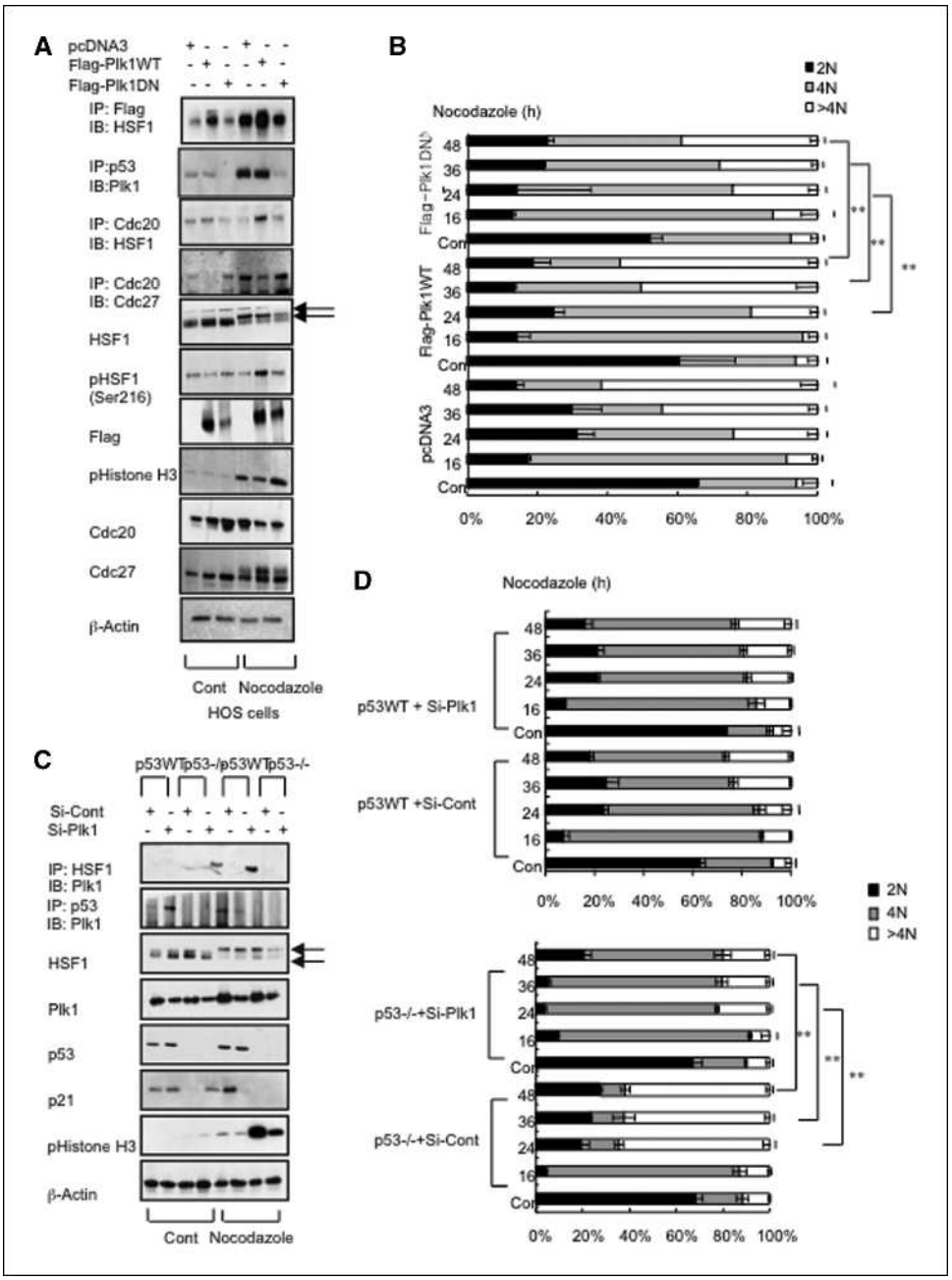

Figure 5. Phosphorylation of Plk1 or p53 by Plk1. *A,* Hos cells were treated with vectors expressing Flag-Plk1 or Plk1DN. Cells were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h) and the lysates were generated for immunoprecipitation and immunoblotting. *B,* the percentage of the DNA content of cells after nocodazole treatment was determined by flow cytometry. *Columns,* mean of three independent experiments; *bars,* SD; **, $P < 0.01$. *C,* p53WT and p53–/– HCT116 cells transiently transfected with control or Si-Plk1 were synchronized in mitosis by treatment with nocodazole (100 nmol/L nocodazole, 16 h). The mitotic cells were harvested and lysates were generated for immunoprecipitation and immunoblotting. *D,* the percentage of the DNA content of cells after nocodazole treatment was determined by flow cytometry. *Columns,* mean of three independent experiments; *bars,* SD; **, $P < 0.01$.

Binding of p53 with Plk1 is essential for the inhibition of aneuploidy production by HSF1. To examine the relationship between endogenous HSF1 expression and production of aneuploidy according to the p53 status, we used cancer cells containing a p53 mutation, HOS cells, and wild-type p53 cancer cells, NCI-H460 cells; both cell types showed a high expression of HSF1. When p53WT plasmid was transfected into HOS cells, the HSF1-Plk1 interaction was inhibited in nocodazole-treated mitotic cells but the p53-Plk1 interaction was increased. Increased HSF1 phosphorylation at Ser216 and the binding activity of HSF1 and Cdc20 in mitotic HOS cells was inhibited by p53WT transfection. Mitotic arrest was also inhibited by p53WT transfection with the detection of phosphorylated histone H3. When NCI-H460 cells were treated with p53 siRNA, the interaction between p53 and Plk1 in mitosis disappeared; however, an HSF1-Plk1 interaction occurred. HSF1 phosphorylation at Ser216 and the binding activity of HSF1 and Cdc20 were restored after transfection with p53 siRNA (Fig. 4*A*). The expression of downstream molecules such as p21 and Bax (Bax data not shown) was not affected following treatment of nocodazole treatment in p53-defective HOS cells and p53 wild-type NCI-H460 cells, which indicates that p53 function in mitosis was independent of p53 transcriptional regulation.

To elucidate whether binding of p53 to Plk1 was essential for inhibition of HSF1 phosphorylation and HSF1-mediated aneuploidy production, deletion mutants of p53 (HA-p53F1 and HA-p53F2) were transfected into HOS or NCI-H460 cells. In HOS cells, HA-p53F1 can bind to Plk1, as the Plk1 binding domain (amino acids 102–292) in the p53 DNA sequence is present. HA-p53F2 cannot bind to Plk1, as the Plk1 binding domain (amino acids 102–292) in the p53 DNA sequence is absent. HA-p53F1 transfection induced

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an interaction of HA-p53F1 with Plk1, whereas binding of HSF1-Plk1 or HSF1-Cdc20 was inhibited, which coincided with the inhibition of phosphorylated HSF1 at Ser216 (Fig. 4*B, top*). Nocodazole-mediated aneuploidy production was significantly inhibited after HA-p53F1 transfection but not HA-p53F2 transfection into HOS cells. These findings suggest that the Plk1-p53 interaction is important to inhibit HSF1-mediated aneuploidy production (Fig. 4*B, bottom*).

Phosphorylation by Plk1 is essential for HSF1- or p53-dependent mitotic regulation. To elucidate whether phosphorylation of p53 or HSF1 by Plk1 in p53 wild-type or defective cells is essential for HSF1- or p53-mediated mitotic regulation and aneuploidy production, HOS cells were transfected with plasmids that expressed Plk1 wild-type (Plk1WT) or a Plk1 dominant-negative mutant (Plk1DN). HSF1 phosphorylation at Ser216 and the binding of Plk1 and HSF1 or the binding of HSF1 and Cdc20 were only observed in nocodazole-treated Plk1WT-transfected cells, whereas the Cdc20-Cdc27 interaction was reduced. However, Plk1DN transfection inhibited these effects (Fig. 5*A*). Nocodazole-mediated aneuploidy in HOS cells was inhibited by Plk1DN transfection but not with Plk1WT transfection, suggesting that Plk1 function is important for HSF1-mediated aneuploidy production (Fig. 5*B*). When p53WT or p53−/− cells were subjected to RNA interference for Plk1 (with Si-Plk1), the increased binding activity of Plk1-HSF1 and HSF1 phosphorylation at Ser216 in mitotic cells was blocked by Si-Plk1 in p53−/− cells. In p53WT cells, a p53-Plk1 interaction was seen and treatment of cells with Si-Plk1 inhibited this interaction (Fig. 5*C*). We also examined these binding interactions in p53 wild-type NCI-H460 cells with or without transfection of Si-p53 (Supplementary Fig. S5*A*). Nocodazole-mediated aneuploidy in p53−/− cells was dramatically inhibited by treatment of cells with Si-Plk1. For p53WT cells, Si-Plk1 treatment did not induce any difference compared with cells treated with a control siRNA (Fig. 5*D*). The downstream effects of p53 such as p21 induction

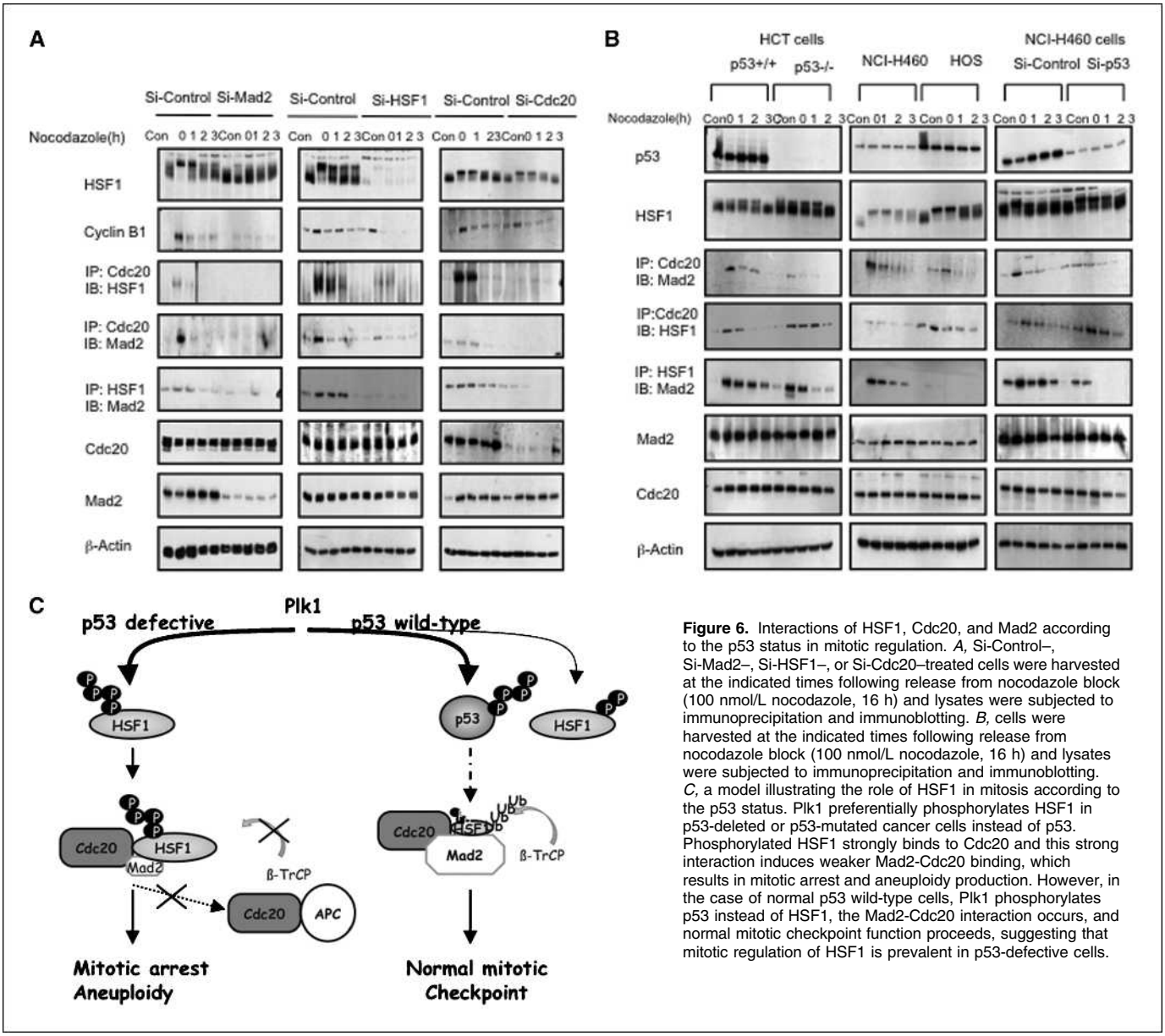

Figure 6. Interactions of HSF1, Cdc20, and Mad2 according to the p53 status in mitotic regulation. *A,* Si-Control–, Si-Mad2–, Si-HSF1–, or Si-Cdc20–treated cells were harvested at the indicated times following release from nocodazole block (100 nmol/L nocodazole, 16 h) and lysates were subjected to immunoprecipitation and immunoblotting. *B,* cells were harvested at the indicated times following release from nocodazole block (100 nmol/L nocodazole, 16 h) and lysates were subjected to immunoprecipitation and immunoblotting. *C,* a model illustrating the role of HSF1 in mitosis according to the p53 status. Plk1 preferentially phosphorylates HSF1 in p53-deleted or p53-mutated cancer cells instead of p53. Phosphorylated HSF1 strongly binds to Cdc20 and this strong interaction induces weaker Mad2-Cdc20 binding, which results in mitotic arrest and aneuploidy production. However, in the case of normal p53 wild-type cells, Plk1 phosphorylates p53 instead of HSF1, the Mad2-Cdc20 interaction occurs, and normal mitotic checkpoint function proceeds, suggesting that mitotic regulation of HSF1 is prevalent in p53-defective cells.

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(Supplementary Fig. S5*A*) and radiation (5 and 10 Gy)–induced apoptosis (Supplementary Figs. S5*B* and *C*) were blocked in p53–/– cells compared with p53WT cells. In addition, Plk1WT transfection to NCI-H460 cells inhibited p53-dependent apoptosis by radiation and transfection with Plk1DN restored radiation-induced apoptosis (Supplementary Fig. S5*D*), suggesting that the Plk1-p53 interaction differently affects mitotic regulation and apoptosis.

Sustained phosphorylation of HSF1 by Plk1 in p53-defective cells induced a stronger interaction with Cdc20, weakening the interaction between Cdc20 and Mad2. Based on preliminary findings, HSF1 bound to Mad2, a mitotic checkpoint molecule (25), suggesting that HSF1 may be involved in spindle checkpoint regulation as a complex of HSF1-Mad2-Cdc20. Indeed, RNA interference studies using Si-HSF1, Si-Mad2, and Si-Cdc20 in HOS cells have revealed the presence of a HSF1-Cdc20-Mad2 complex in mitosis (Fig. 6*A*; Supplementary Fig. S6*A*). When we examined colocalization between HSF1 and Cdc20 or HSF1 and Mad2 in the mitosis period of HOS cells, colocalization between two molecules was detected (Supplementary Fig. S6*B*). Knockdown of each protein inhibited binding activities among the three proteins, which suggested the presence of a three-protein complex of HSF1-Cdc20-Mad2 in mitosis. Next, we examined binding affinity among HSF1, Cdc20, and Mad2 in p53 wild-type and p53-defective cells. The binding affinity of HSF1-Cdc20 was stronger than the binding affinity of Mad2-Cdc20 in p53-deleted or p53-mutated cells, whereas for p53 wild-type cells, the Mad2-Cdc20 interaction was stronger than the HSF1-Cdc20 interaction (Fig. 6*B*). From the results, it could be suggested that mitotic regulation of HSF1 was prevalent in p53-defective cells.

Discussion

In this study, we have investigated the roles and mutual relationships between HSF1, Plk1, and p53. We found that HSF1-mediated mitotic arrest and aneuploidy production are p53 dependent. In cells with wild-type p53, Plk1 phosphorylated p53 and proper mitotic regulation such as mitotic checkpoint function occurred. However, in cancers cells with a defective p53 and higher expression of HSF1, Plk1 phosphorylated HSF1 at Ser216 instead of p53, a tight interaction of HSF1 and Cdc20 was induced, and defective mitotic regulation and aneupoloidy occurred. Moreover, binding of phosphorylated HSF1 and Cdc20 inhibited the Mad2-Cdc20 interaction, which resulted in abnormal mitotic checkpoint function and increased mitotic arrest and aneuploidy in p53-defective cells. In the case of p53 wild-type cells, HSF1 phosphorylation was inhibited and a stronger Mad2-Cdc20 interaction occurred with normal mitotic checkpoint function.

A previous report has shown that overexpression of dominant-negative HSF1 inhibited aneuploidy and this effect was mediated by the delayed breakdown of cyclin B1 (29). Our previous findings also indicate that HSF1 directly binds to Cdc20 and the prolonged interaction of HSF1 and Cdc20 in cells that overexpress HSF1 induces mitotic arrest that is independent of transcriptional activity, resulting in aneuploidy production (23). We have also identified that HSF1 is an essential checkpoint component that resides at the kinetochore during mitosis. Moreover, the timing regulation of HSF1 seems to be important for regulation of mitosis and overexpressed HSF1 and prolonged binding of HSF1 and Cdc20, which is usually shown in cancer cells, can induce mitotic arrest and aneuploidy production (23).

A recent report has suggested that a selective decrease of several cancers occurs by functional loss of *Hsf1* in a p53-deficient mouse model (30). Therefore, HSF1 might be involved in tumorigenesis, especially under conditions where p53 is defective. In addition, the elimination of HSF1 was shown to protect mice from tumors induced by mutations of the Ras oncogene or a hotspot mutation in p53 (19). HSF1 has been shown to support malignant transformation by orchestrating a network of core cellular functions, including proliferation, survival, protein synthesis, and glucose metabolism in mouse and human systems (19). In the present study, we also examined that HSF1 regulation in mitosis (improper segregation and aneuploidy production) was mostly observed in p53-defective cancer cell lines and not in p53 wild-type cells (Fig. 1). Prolonged expression of cyclin B1 and securin was observed coincident with increased phosphorylation at Ser216 of HSF in p53-defective cancer cells compared with p53 wild-type cells. However, when the p53 status was restored to normal in p53-defective cells, HSF1-mediated mitotic dysregulation such as aneuploidy production disappeared. Induction of the binding activity of HSF1 with Cdc20 in p53-defective cells was abolished when p53 function was restored (Fig. 2). From these results, it was shown that HSF1-mediated mitotic arrest and aneuploidy production was dependent on the p53 status. Localization of HSF1 at the spindle pole was always observed in both p53WT and p53–/– cells, suggesting that defective mitotic progression in p53–/– cells was due to the abnormal transition from metaphase to anaphase. Therefore, it can be hypothesized that in p53–/– cells, HSF1 phosphorylation by Plk1 occurs, and phosphorylated HSF1 directly binds to Cdc20 and delays HSF1 phosphorylation and sustains an interaction with Cdc20 that blocks the recruitment of β-TrCP and HSF1 degradation, resulting in a defective transition from metaphase to anaphase.

In a previous report, the physical and functional interaction between Plk1 and p53 was shown to inhibit p53-dependent transcriptional activation as well as its proapoptotic function (16). In our study, Plk1 interacted with p53 in p53 wild-type cells, which inhibited HSF1-mediated aneuploidy production (Fig. 3). For this function, a binding domain of p53 for interaction with Plk1 is required (Fig. 4). The Plk1-p53 interaction inhibited p53-mediated p21 expression and apoptosis in p53 wild-type cells (Fig. 5) as has been described previously (16). Actually, Plk1 inhibited p53-dependent apoptosis (Supplementary Fig. S5). From these findings, it can be postulated that the interaction of Plk1 with p53 is not a positive function for both the transcriptional activity and apoptotic function. In the case of mitotic regulation such as with the interaction with Plk1, p53 function was differently involved. The Plk1-p53 interaction in p53 wild-type cells showed a normal mitotic checkpoint such as for the inhibition of aneuploidy induced by nocodazole treatment. However, as HSF1 is overexpressed in p53-defective cancer cells, the Plk1-HSF1 interaction was dominant instead of the Plk1-p53 interaction, which resulted in delayed phosphorylation of HSF1 at Ser216. Phosphorylated HSF1 leads to the binding of Cdc20-HSF1 and the prolonged binding of Cdc20-HSF1 increased aneuploidy production.

Because previous studies have shown that Mad2 is a major mitotic checkpoint protein for the interaction with Cdc20 and from our findings, HSF1 was found to be co-complexed with Cdc20-Mad2 (Fig. 6). The binding site of Cdc20 to interact with HSF1 or Mad2 is same sequence (amino acids 106–171), which indicated a possibility of competition between HSF1 and Mad2 to interact with Cdc20 (23). Indeed, the binding affinity of the HSF1-Cdc20

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was stronger than the Mad2-Cdc20 interaction in p53-defective cells; however, the Mad2-Cdc20 interaction was stronger than the HSF1-Cdc20 interaction in p53 wild-type cells. From the findings, it can be hypothesized that p53-defective cells inhibit the normal binding between Mad2-Cdc20 that shows a normal checkpoint function in p53 wild-type cells because of the prolonged binding between HSF1-Cdc20, resulting in prolonged mitotic arrest and aneuploidy production.

Figure 6*C* shows a model that incorporates our present findings and illustrates interactions among p53; HSF1 and Plk1; and HSF1, Cdc20, and Mad2 in mitotic regulation. In most cancer cells, p53 function is defective and Plk1 activity is high. Therefore, under these conditions, HSF1 overexpression may facilitate mitotic dysregulation such as increased mitotic arrest and aneuploidy production through increased HSF1 phosphorylation by Plk1 and the prolonged interaction of HSF1 and Cdc20, as well as inhibit the interaction between HSF1 and Mad2. Therefore, the function of HSF1 in mitotic regulation may be more deleterious to cancer cells and HSF1 may be one of many targets to understand cancer cell physiology. As p53 is defective in >50% of tumors, the assertion that p53 has an important role in the prevention of tumor development is supported, especially in HSF1-overexpressed cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

1. Vogel C, Kienitz A, Hofmann I, Muller R, Bastians H. Crosstalk of the mitotic spindle assembly checkpoint with p53 to prevent polyploidy. Oncogene 2004;23:6845–53.
2. Lanni JS, Jacks T. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. Mol Cell Biol 1998;18:1055–64.
3. Michel LS, Liberal V, Chatterjee A, et al. MAD2 haploinsufficiency causes premature anaphase and chromosome instability in mammalian cells. Nature 2001;409:355–9.
4. Minn AJ, Boise LH, Thompson CB. Expression of Bcl-xL and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. Genes Dev 1996;10:2621–31.
5. Tritarelli A, Oricchio E, Ciciarello M, et al. p53 localization at centrosomes during mitosis and postmitotic checkpoint are ATM-dependent and require serine 15 phosphorylation. Mol Biol Cell 2004;15:3751–7.
6. Ciciarello M, Mangiacasale R, Casenghi M, et al. p53 displacement from centrosomes and p53-mediated G1 arrest following transient inhibition of the mitotic spindle. J Biol Chem 2001;276:19205–13.
7. Gualberto A, Aldape K, Kozakiewicz K, Tlsty TD. An oncogenic form of p53 confers a dominant, gain-of-function phenotype that disrupts spindle checkpoint control. Proc Natl Acad Sci U S A 1998;95:5166–71.
8. Cross SM, Sanchez CA, Morgan CA, et al. A p53-dependent mouse spindle checkpoint. Science 1995;267:1353–6.
9. Shi Q, King RW. Chromosome nondisjunction yields tetraploid rather than aneuploid cells in human cell lines. Nature 2005;437:1038–42.
10. Fujiwara T, Bandi M, Nitta M, Ivanova EV, Bronson RT, Pellman D. Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. Nature 2005;437:1043–7.
11. Lake RJ, Jelinek WR. Cell cycle- and terminal differentiation-associated regulation of the mouse mRNA encoding a conserved mitotic protein kinase. Mol Cell Biol 1993;13:7793–801.
12. Golsteyn RM, Schultz SJ, Bartek J, Ziemiecki A, Ried T, Nigg EA. Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila polo* and *Saccharomyces cerevisiae* Cdc5. J Cell Sci 1994;107:1509–17.
13. Golsteyn RM, Mundt KE, Fry AM, Nigg EA. Cell cycle regulation of the activity and subcellular localization of Plk1, a human protein kinase implicated in mitotic spindle function. J Cell Biol 1995;129:1617–28.
14. Hamanaka R, Smith MR, O'Connor PM, et al. Polo-like kinase is a cell cycle-regulated kinase activated during mitosis. J Biol Chem 1995;270:21086–91.
15. Qian YW, Erikson E, Maller JL. Purification and cloning of a protein kinase that phosphorylates and activates the polo-like kinase Plx1. Science 1998;282:1701–4.
16. Ando K, Ozaki T, Yamamoto H, et al. Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and phosphorylation. J Biol Chem 2004;279:25549–61.
17. Liu X, Erikson RL. Polo-like kinase (Plk)1 depletion induces apoptosis in cancer cells. Proc Natl Acad Sci U S A 2003;100:5789–94.
18. Xie S, Wu H, Wang Q, et al. Plk3 functionally links DNA damage to cell cycle arrest and apoptosis at least in part via the p53 pathway. J Biol Chem 2001;276:43305–12.
19. Dai C, Whitesell L, Rogers AB, Lindquist S. Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. Cell 2007;130:1005–18.
20. Westerheide SD, Morimoto RI. Heat shock response modulators as therapeutic tools for diseases of protein conformation. J Biol Chem 2005;280:33097–100.
21. Christians ES, Yan LJ, Benjamin IJ. Heat shock factor 1 and heat shock proteins: critical partners in protection against acute cell injury. Crit Care Med 2002;30:S43–50.
22. Solimini NL, Luo J, Elledge SJ. Non-oncogene addiction and the stress phenotype of cancer cells. Cell 2007;130:986–8.
23. Lee YJ, Lee HJ, Lee JS, et al. A novel function for HSF1-induced mitotic exit failure and genomic instability through direct interaction between HSF1 and Cdc20. Oncogene 2008;27:2999–3009.
24. Tang D, Khaleque MA, Jones EL, et al. Expression of heat shock proteins and heat shock protein messenger ribonucleic acid in human prostate carcinoma *in vitro* and in tumors *in vivo*. Cell Stress Chaperones 2005;10:46–58.
25. Lee YJ, Kim EH, Lee JS, et al. HSF1 as a mitotic regulator: phosphorylation of HSF1 by Plk1 is essential for mitotic progression. Cancer Res 2008;68:7550–60.
26. Soh JW, Lee EH, Prywes R, Weinstein IB. Novel roles of specific isoforms of protein kinase C in activation of the c-fos serum response element. Mol Cell Biol 1999;19:1313–24.
27. Lee YJ, Lee DH, Cho CK, et al. HSP25 inhibits protein kinase C δ-mediated cell death through direct interaction. J Biol Chem 2005;280:18108–19.
28. Hans F, Dimitrov S. Histone H3 phosphorylation and cell division. Oncogene 2001;20:3021–7.
29. Wang Y, Theriault JR, He H, Gong J, Calderwood SK. Expression of a dominant negative heat shock factor-1 construct inhibits aneuploidy in prostate carcinoma cells. J Biol Chem 2004;279:32651–9.
30. Min JN, Huang L, Zimonjic DB, Moskophidis D, Mivechi NF. Selective suppression of lymphomas by functional loss of Hsf1 in a p53-deficient mouse model for spontaneous tumors. Oncogene 2007;26:5086–97.

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