Negative Feedback Regulation of Aurora-A via Phosphorylation of Fas-associated Factor-1*‡

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This study reports that Aurora-A (Aur-A) phosphorylates Fas-associated factor-1 (FAF1) at Ser-289 and Ser-291. Forced expression of a FAF1 mutant mimicking phosphorylation at Ser-289 and Ser-291 (FAF1 DD), but not phosphorylation-deficient FAF1 (FAF1 AA), reduced Aur-A expression. However, transfection of FAF1 DD failed to reduce Aur-A expression in the presence of MG132 and MG115, indicating that this decrease is proteasome-mediated. Additionally, transfection of FAF1 DD suppressed the expression of Aur-A in ts20-BALB cells lacking E1 ubiquitin (Ub) activating enzyme activity at restrictive temperatures and also reduced the expression of Aur-A S51D, a mutant resistant to Ub-dependent degradation. Our data indicate that phosphorylated FAF1 mediates the ubiquitin-independent, proteasome-dependent degradation of Aur-A. Overexpression of FAF1 DD blocked Aur-A-induced centrosome amplification and accumulated cells in G2/M phase, representing cellular phenotypes consistent with the anticipated loss of Aur-A. Collectively, our findings support the negative feedback regulation of Aur-A via phosphorylation of the death-promoting protein, FAF1, and disclose the presence of molecular cross-talk between constituents of the cell cycle and cell death machinery.

Aurora-A (Aur-A)3 is amplified in a variety of primary human tumors, including gastric, breast, and colorectal cancers, implying involvement in oncogenesis and tumor progression (1–4). Increased Aur-A expression induces centrosome amplification, chromosome instability, mitotic abnormalities, and aneuploidy leading to malignant transformations in experimental rodent models (5–8).

Aur-A expression is carefully regulated for ordered progression of the cell cycle. The protein is degraded via both ubiquitin (Ub)-dependent and -independent proteolysis steps. Ub-dependent degradation of Aur-A occurs in a cell cycle-dependent manner and is mediated by E3 ligase, the APC (anaphase-promoting complex) (9). Degradation of Aur-A is additionally regulated by the phosphorylation status of Ser-51 residue (10). Specifically, protein phosphatase 2A dephosphorylates Ser-51, consequently promoting degradation (11). In contrast, cell cycle-independent degradation of Aur-A occurs through antizyme1, a mediator of the Ub-independent protein degradation (12). Aur-A kinase interacting protein 1 (AURKAIP) seems to promote the antizyme1-mediated degradation (13) even though its effects and underlying mechanisms are unclear at present (14).

Fas-associated factor-1 (FAF1) promotes cell death through several mechanisms. For instance, FAF1 enhances caspase-8 assembly in response to the Fas signal (15) and potentiates death effector filament formation after treatment with chemotherapeutics (16). Other studies show that FAF1 suppresses nuclear factor κB (NF-κB) activation by disrupting IκB kinase complex assembly and preventing nuclear translocation of NF-κB RelA (p65) in a stimulation-dependent manner (17, 18). Moreover, FAF1 inhibits the chaperone activities of valosin-containing protein and Hsp70 (19, 20). Phosphorylation of Faf1 at Ser-289 and Ser-291 has been reported (21). The phosphorylation status of FAF1 does not seem to affect Fas signaling (22) but sensitizes cells to various DNA-damaging agents (23). The mechanisms and physiological implications of FAF1 phosphorylation await further investigation.

In this study we demonstrate that Aur-A phosphorylates FAF1. The phosphorylated FAF1, in turn, mediates the Ub-independent, proteasome-dependent degradation of Aur-A, representing a negative feedback regulatory mechanism. Our data also provide an example of a cell cycle promoting kinase performing an anti-proliferative role.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Treatments**—Stable Aur-A knock-down cell lines were generated by transfecting HeLa cells with pSuper-
Plasmid Construction, Mutagenesis, and RNA Interference—

FAF1 and its deletion mutants were generated in the pGex 4T-1 vector using standard PCR techniques employing custom-designed primers containing appropriate restriction site. Aur-A and its deletion constructs were cloned into the FLAG vector. Purified GST-tagged Aurora kinases were purchased from MBL (Hamamatsu, Japan). The FAF1 substitution mutants S289A, S291A, S289A/S291A, and S289D/S291D were generated using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). Aur-A siRNA targeting the sequence 5’-ATTCCTCAGCGGTTC3’ and scrambled siRNA were provided by Qiagen Inc. (Valencia, CA). For Aur-A knockdown stable cell line, the pSuper-puro-Aur-A siRNA plasmid was generated using the Aur-A siRNA targeting the sequence 5’-ACAAGCCGGTTCAGAATCA-3’. The pSuper-puro vector was purchased from Oligoengine (Seattle, WA).

Antibodies—Mouse anti-FAF1 antibody was provided by Dr. Jong-Seok Lim (Sookmyung Women’s University, Seoul, Korea). Anti-P-FAF1 antibody was raised in a rabbit against a synthetic peptide corresponding to residues 284–295 of human FAF1 (phosphorylated at Ser-289 and Ser-291) and further purified by affinity chromatography (AbFrontier, Seoul, Korea). Anti-FLAG, anti-α-tubulin, and anti-mouse TRITC-conjugated IgG antibodies were obtained from Sigma. Anti-Aur-A and anti-p53 antibodies were purchased from Santa Cruz Technology (Santa Cruz, CA). The anti-GAPDH antibody was acquired from AbFrontier.

Immunoprecipitation—HeLa cells were either treated with taxol or left untreated. Cells were lysed in mammalian lysis buffer (17), sonicated, and centrifuged at 13,000 rpm for 10 min at 4 °C. Cell lysates were incubated for 6 h at 4 °C with anti-FAF1 or anti-Aur-A antibody and protein A/G-Sepharose beads (Santa Cruz Technology) and immunoprecipitated. Immunoprecipitates and whole cell lysates were subjected to SDS-PAGE, separated, transferred to a nitrocellulose membrane, and immunoblotted with the indicated antibodies.

Glutathione S-Transferase Pulldown Assay—For identification of the FAF1 domain interacting with Aur-A, GST full-length FAF1 and truncated mutant proteins were purified from bacteria using glutathione-Sepharose beads (17). GST fusion proteins were incubated with taxol-treated HeLa cell lysates for 4 h at 4 °C. Samples were washed with lysis buffer. Bound proteins were separated by SDS-PAGE and detected by immunoblotting with an anti-Aur-A antibody. To determine the Aur-A domain interacting with FAF1, HeLa cells were transfected with FLAG-tagged full-length Aur-A and deletion constructs using METAFFECTENE (Biontex, Munich, Germany) according to the manufacturer’s protocol. At 36 h after transfection, cells were treated with taxol (500 nM) for 12 h. Cell lysates were incubated with GST-FAF1 fusion proteins for 4 h at 4 °C. After extensive washing with lysis buffer, samples were subjected to SDS-PAGE, separated, and immunoblotted with an anti-FLAG antibody.

In Vitro Kinase Assay—HeLa cells were transfected with FLAG-tagged FAF1 wild-type (WT) and mutants. 36 h after transfection, cells were treated with taxol for 12 h. Whole cell lysates were incubated with anti-Aur-A antibody and protein A/G-Sepharose beads at 4 °C for 6 h. Immunoprecipitated Aur-A proteins were incubated with myelin basic protein (Sigma) in kinase buffer (20 mM HEPES-KOH, pH 7.5, 5 mM MgCl₂, 1 mM dithiothreitol, 1 μCi of [γ-32P]ATP, 100 mM ATP). After 30 min at 30 °C, the reactions were mixed with equal volumes of protein loading buffer, and the phosphorylated substrates were detected by SDS-PAGE and autoradiography. For phosphorylation site mapping of FAF1, recombinant GST-tagged FAF1 were incubated with GST-tagged recombinant Aur-A in kinase buffer coupled with 1 μCi of [γ-32P]ATP at 30 °C for 30 min. After reaction, the bound proteins were analyzed by SDS-PAGE.

Gel Filtration Chromatography—HeLa cells were transfected with 5 μg of vector, FLAG-FAF1 AA, or FAF1 DD. Cells were lysed in a buffer (20 mM Tris-Cl, pH 7.4, 1 mM phenylmethylsulfonyl fluoride, and 0.1% CHAPS) and clarified by centrifugation at 30,000 rpm for 30 min at 4 °C. Cell extracts were fractionated on a Superose 6 column with an A¨ KTA fast protein liquid chromatography system (GE Healthcare). 2 ml of cell lysates (3 mg of total proteins) was loaded onto the column and separated at a flow rate of 0.5 ml/min. The molecular mass standards (GE Healthcare) used to calibrate the column were blue dextran (2000 kDa), thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), albumin (67 kDa), and ribonuclease A (13 kDa).

Immunofluorescence Staining of Centrosomes—HeLa cells transfected with the indicated plasmids and GFP were placed on sterile coverslips within 6-well plates. After 12 h, cells were fixed with 3.7% formaldehyde in PBS for 15 min at room temperature and washed with PBS. Cells were incubated with anti-α-tubulin antibody for 45 min, washed with PBS, and treated with TRITC-conjugated goat anti-mouse IgG antibody for 30 min. Cells were washed three times with PBS and mounted on slides. Color images were acquired using a Bio-Rad Radiance 2000 laser scanning confocal microscope (Bio-Rad). Centrosomes were visualized by staining with the anti-α-tubulin antibody and counted. Centrosomes of GFP-positive cells were randomly selected.

Flow Cytometry—Cells were co-transfected with GFP and FLAG-FAF1 mutants. After 24 h HeLa cells were treated with aphidicolin (1 μg/ml) for 13 h, released by washing, and maintained in fresh medium. 13 h after release from the aphidicolin block, cells were assessed by propidium iodide (PI) staining and flow cytometry (FACSCalibur, BD Biosciences). Briefly, cells were harvested and fixed with 70%
ethanol overnight at −20 °C. Fixed cells were washed twice in PBS, resuspended in PI staining solution (50 μg/ml PI, 100 μg/ml RNase), and incubated for 1 h at 37 °C before analysis. The GFP-positive cell population was determined by FACS analysis.

RESULTS

Interactions between FAF1 and Aur-A—To elucidate the molecular mechanisms underlying reduced FAF1 expression in gastric tumor tissues (24), we screened for FAF1-interactors among cDNAs displaying differential expression patterns in gastric cancer tissues of Korean patients when compared with their normal counterparts. Briefly, differentially expressed genes in Korean gastric cancer tissues were translated in vitro, and the GST pulldown assay was performed between GST-tagged FAF1-DEDID (death effector domain; residues 181–381), which is essential for cell death mediation (25) and the in vitro translated genes. Several proteins interacting with FAF1-DEDID were identified, including Aur-A.

The Aur-A expression level was examined in the Korean gastric cancer tissues. Aur-A expression profiles of matching gastric tumor and normal tissues of 57 Korean patients were analyzed by immunohistochemistry. We graded the staining intensity as the following: negative (score 0), weak (score 1), moderate (score 2), and strong (score 3) (supplemental Fig. S1A). A significant proportion of gastric tumor tissue specimens (42/57, 73.7%) showed moderate to strong Aur-A staining (score 2 and 3). In contrast, only 7 in 57 samples (12.3%) showed moderate Aur-A staining in gastric normal tissues. No gastric normal tissue demonstrated strong Aur-A staining. Therefore, our data clearly show that Aur-A expression is elevated in the cancer tissues of Korean gastric cancer patients.

Next, the interaction between FAF1 and Aur-A was examined in the gastric tumors. Consistent with the previous studies, decreased expression of FAF1 and increased expression of Aur-A were observed in the gastric tumor tissues (3, 24). Aur-A immunoprecipitates were prepared from two matching gastric normal and tumor tissues and immunoblotted with the anti-FAF1 antibody. Binding of Aur-A to FAF1 was significantly increased in the gastric tumor tissues when compared with that in the gastric normal tissues (supplemental Fig. S1B). Therefore, FAF1-Aur-A interaction occurs in the clinical situation.

FAF1-Aur-A interactions were further examined in HeLa cells (Fig. 1A). Immunoprecipitation and subsequent immunoblotting with the anti-FAF1 or anti-Aur-A antibody disclosed that FAF1 and Aur-A did not interact in untreated HeLa cells. However, FAF1 and Aur-A formed complexes in taxol-treated HeLa cells. Moreover, interaction between FAF1 and Aur-A were induced in diverse stress such as etoposide, doxorubicin, and camptothecin (data not shown), implying that interactions between these proteins are enhanced in a stimulus-dependent manner.

To determine the FAF1 domain responsible for binding to Aur-A, the GST pulldown assay was performed using GST-
Aur-A phosphorylates FAF1. A, taxol-treated (500 nM, 12 h) HeLa cell lysates were incubated for 1 h with 1000 units of λ-protein phosphatase (λ-ppase) at 30 °C and analyzed by immunoblotting (WB) with the indicated antibodies (left panels). HeLa cells were transiently transfected with 2 μg of either scrambled siRNA (scRNA) or Aur-A targeting siRNA (siAur-A). 36 h after transfection, cells were subsequently treated with taxol (500 nM for 12 h). Transfected cells were harvested for immunoblotting using the indicated antibodies (middle panels). For stable depletion of Aur-A, HeLa cells were transfected with control pSuper-puro (pS) or pSuper-puro-Aur-A siRNA (pS-Aur-A) vectors and selected with puromycin. Selected cells were treated with taxol (500 nM for 12 h) and harvested and processed for immunoblotting with the indicated antibodies (right panels). B, HeLa cells were left untreated (Emp) or treated with the VX680 (3 μM) for 12 h with taxol (500 nM). Cells treated with DMSO were used as the control (Veh). VX680 was employed as the Aurora kinase inhibitor. Stimulated cells were harvested for immunoblotting using anti-Aur-A, anti-P-FAF1, or anti-GAPDH antibody. C, purified GST-Aur-A proteins were incubated with purified GST or GST-FAF1 with or without VX680 (1 μM) in the presence of [γ-32P]ATP. GST proteins were resolved by SDS-PAGE and visualized by autoradiography (for the kinase assay) or Coomassie Blue staining. D, GST, GST-Aur-A WT, and mutant proteins (GST-Aur-A 289A/S291A, GST-Aur-A 5291A, or GST-Aur-A 289A/291A) were incubated with GST-Aur-A and [γ-32P]ATP. Proteins were resolved and visualized by autoradiography (for the kinase assay) or Coomassie Blue staining. The results shown are representatives of at least three independent experiments.

Aur-A Phosphorylates FAF1 at Ser-289 and Ser-291—Next, we investigated whether FAF1-Aur-A interactions were associated with the phosphorylation of FAF1. Anti-Aur-A antibody detected two bands in taxol-treated HeLa cells (Fig. 2A). To determine whether the upper band is the phosphorylated protein, we incubated the taxol-treated HeLa cell lysates with the λ-protein phosphatase. λ-protein phosphatase eradicated the upper band, implying that it is the phosphorylated FAF1 (P-FAF1). Next, we have raised a polyclonal antibody against a synthetic peptide corresponding to residues 284–295 of FAF1 (phosphorylated at Ser-289 and Ser-291) (anti-P-FAF1 antibody), based on the previous identification of Ser-289 and Ser-291 as phosphorylation sites of FAF1 using mass spectrometry analysis (21). The presence of P-FAF1 was confirmed by anti-P-FAF1 antibody (Fig. 2A, left panel). Next, we examined whether Aur-A is responsible for the phosphorylation of FAF1. Cells were transfected with Aur-A-specific siRNA (siAur-A) for transient depletion or vector-based siRNA against Aur-A (pSuper-puro-Aur-A) followed by selection with puromycin for stable depletion. FAF1 phosphorylation was clearly inhibited in cells both transiently and stably depleted of Aur-A (Fig. 2A) in taxol-treated HeLa cells. The addition of VX680, a specific Aurora kinase inhibitor, to taxol-treated HeLa cells also suppressed phosphorylation of FAF1 (Fig. 2B).

The Aur-A kinase assay was performed in vitro using GST-FAF1 as the substrate. Aur-A, but not Aur-B or Aur-C, phosphorylated GST-FAF1 in vitro, implying that FAF1 is an Aur-A-specific substrate (supplemental Fig. S2). Phosphorylation was blocked by treatment with VX680 (Fig. 2C). To assess whether Ser-289 and Ser-291, previously identified phosphorylation sites (21), are phosphorylated by Aur-A, GST fusion proteins of single (289A or 291A) and double (289A/291A) FAF1 substitution mutants were generated. Single substitutions (289A and 291A) failed to block FAF1 phosphorylation completely. Notably, the mutation at position 291 inhibited Aur-A phosphorylation to a greater extent than at 289. In contrast, double substitution (289A/291A) abolished FAF1 phosphorylation almost completely (Fig. 2D). Our results indicate that both serine residues of FAF1 are phosphorylated by Aur-A. It is worth mentioning that these serines are not embedded in the consensus motif of Aur-A substrates, (R/K)X_{1–3}(S/T) (26). Phosphorylations of proteins by Aur-A without the consensus motif have been shown in p53 and BRCA1 (27, 28).

P-FAF1 Mediates the Ub-independent, Proteasome-dependent Degradation of Aur-A—To explore the implications of the interaction between FAF1 and Aur-A, we overexpressed FAF1 and Aur-A in HeLa cells. Overexpression of FAF1 and Aur-A allowed their interactions without taxol treatment (data not shown). HeLa cells transfected with FAF1 WT, FAF1 AA (FAF1
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S289A/S291A), and FAF1 DD (FAF1 S289D/S291D) displayed differential Aur-A expression patterns. Aur-A expression was significantly decreased in cells transfected with FAF1 WT or FAF1 DD in a dose-dependent manner but not FAF1 AA (Fig. 3A and supplemental Fig. S3). Consistent with these findings, Aur-A kinase activity was reduced in cells transfected with FAF1 WT and FAF1 DD (supplemental Fig. S3). These results demonstrate that the phosphorylation status of FAF1 regulates Aur-A expression. The depletion of FAF1 increased the protein level of Aur-A when taxol was treated (supplemental Fig. S4). Concomitant with the increased level of Aur-A, the kinase activity of Aur-A was also increased in the FAF1-depleted cells.

Next, we asked whether P-FAF1 reduces Aur-A expression via regulation of the protein degradation pathway. The decrease in Aur-A expression in FAF1 DD-transfected HeLa cells was significantly inhibited after treatment with the proteasome inhibitors, MG132 and MG115 (Fig. 3B). Previous studies show that Aur-A is degraded both via Ub-dependent and -independent pathways (12, 13). Accordingly, we asked through which pathway FAF1-mediated Aur-A decreases. We examined whether degradation of Aur-A occurs in ts20-BALB cells lacking E1 Ub-activating enzyme activity at the restrictive temperatures (29). In ts20-BALB cells, FAF1 DD mediated Aur-A degradation at the restrictive temperature (39 °C) as effectively as that at the permissive temperature (35 °C), whereas the p53 level remained unchanged at 39 °C (Fig. 3C). Moreover, expression of the Aur-AS51DmutantresistanttoUb-dependent degradation (10) was suppressed by transfection of FAF1 DD (Fig. 3D). Our data indicate that the FAF1-mediated Aur-A decrease occurs through an Ub-independent, proteasome-mediated degradation pathway.

Next, we examined whether the presence of P-FAF1 influences the protein complexes containing Aur-A. HeLa cell lysates were prepared from transfectants containing the vector control (MOCK), FLAG-FAF1 AA, or FLAG-FAF1 DD and subjected to gel filtration chromatography on a Superose 6 column. Aur-A exhibited a broad size distribution, roughly corresponding to 2000 to 67 kDa in the MOCK- and FLAG-FAF1 AA-transfected HeLa cells. Transfection of FLAG-FAF1 DD markedly decreased Aur-A expression when compared with MOCK-or FLAG-FAF1 AA-transfections, especially in the protein complexes with molecular weight of ~440 to 67 kDa. In contrast, Aur-A in the high molecular mass complex (~2000 kDa) was decreased by FLAG-FAF1 DD-transfection but not by FLAG-FAF1 AA-transfection. However, molecular mechanism and biological implication of the differential protection of Aur-A in the molecular mass complex (~2000 kDa) by FAF1 AA-or FAF1 DD-transfection needs further investigation.

FAF1 Antagonizes Aur-A-induced Centrosome Amplification—Overexpression of Aur-A is associated with centrosome abnormalities (30). Thus, the physiological implications of FAF1-Aur-A interactions were further examined in HeLa cells. Specifically, HeLa cells were transfected with FLAG-tagged FAF1 WT, AA, and DD, respectively, together with FLAG-tagged Aur-A and GFP, and centrosome copies were analyzed...
at 48 h post-transfection. Aur-A-transfected HeLa cells exhibited centrosome amplification, as reflected by the findings that 52.4 ± 4.9% of cells expressing Aur-A contained at least three copies of the centrosome (Fig. 4). However, the population of HeLa cells with three or more copies of centrosome was reduced to 28.2 ± 2.1 and 16.8 ± 0.4% upon co-transfection of FLAG-tagged Aur-A with FAF1 WT and FAF1 DD, respectively. Consistently, transfection of FAF1 AA reduced centrosome amplification to a much lower degree (43.2 ± 3.3%). These findings indicate that P-FAF1 antagonizes centrosome amplification induced by Aur-A.

Phosphorylation of FAF1 Induces G2/M Arrest—Next, we investigated the biological effects of FAF1 phosphorylation on G2/M progression in which Aur-A plays an essential role. To that end, HeLa cells were transiently transfected with FLAG-tagged FAF1 WT, AA, or DD, and cell cycle profiles of each transfectant were determined by FACS analyses after 13 h release from the aphidicolin block. The increase of cells in the G2/M phase with a parallel depletion of cells in the G0/G1 phase was observed in FAF1 WT- and FAF1 DD-transfected cells when compared with that in MOCK-transfected cells (Fig. 5). However, accumulation of cells in the G2/M phase in FAF1 AA-transfected cells was much less than those in FAF1 WT and DD-transfected cells. Our data collectively demonstrate that P-FAF1 induces the cell cycle arrest in G2/M phase.

DISCUSSION

In this study we show that FAF1, a novel substrate of Aur-A, functions as an endogenous negative regulator of Aur-A. The enhanced interactions in the gastric tumor tissues between Aur-A and FAF1 suggest that the functional interplay might be more significant under pathological conditions. Our findings provide a molecular explanation for the transition
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from cell cycle arrest to cell death in the response to stimulus.

Aur-A is a well characterized oncoprotein that is highly expressed in various cancers (1–4). In this regard, regulation of the Aur-A level is important in maintaining normal cellular physiology. Negative regulators of Aur-A target its degradation mechanisms. For instance, protein phosphatase 2A promotes degradation of Aur-A through the ubiquitination pathway (10). Antizyme1 mediates, and AURKAIP1 (Aur-A kinase interacting protein 1) potentiates Ub-independent degradation of Aur-A even though the effects of AURKAIP are somewhat controversial (12–14). Our results demonstrate that FAF1 also mediates degradation of Aur-A independently of Ub. The main difference between FAF1 and other negative regulators against Aur-A is that FAF1 is a direct substrate of Aur-A, and thus, suppression of Aur-A via its own substrate establishes an autoregulatory feedback.

FAF1 interacts with valosin-containing protein, the multiubiquitin chain-targeting factor, and inhibits the degradation of ubiquitinated proteins via a proteasome-mediated pathway (19). Our data show that FAF1 is involved in the other branch of Aur-A degradation, specifically the Ub-independent pathway. FAF1 may inhibit Ub-dependent, but potentiate Ub-independent degradation of certain proteins. It is tempting to speculate that FAF1 functions as a switch to turn off the Ub-dependent pathway and turn on the Ub-independent system, depending on the situation. At present, the mechanism by which FAF1 enhances Ub-independent protein degradation and other proteins whose degradation is regulated by FAF1 remains to be established.

It is worth mentioning that the degradation of Aur-A mainly occurred in the protein complexes with molecular masses of ~440 to 67 kDa. The reason for the persistence of Aur-A in the complex with a molecular mass of ~2000 kDa upon FAF1 DD-transfection is not yet clear. Considering that transfection of FAF1 DD antagonizes canonical functions of Aur-A such as centrosome amplification and cell cycle progression, Aur-A in the complex with a molecular mass of ~2000 kDa might perform novel function distinguished from the known ones. Further study would be necessary to elucidate the function of Aur-A in the high molecular weight complex.

Interactions between FAF1 and binding partners in cells are often triggered in response to stimuli, such as Fas and tumor necrosis factor-α (15, 17, 18). In untreated cells, FAF1 may not be freely accessible due to specific masking proteins that prevent interactions with other proteins. Hsp70 is a possible candidate for the masking role, as it interacts with FAF1 in untreated cells (20). Hsp70 acts as a masking protein for death proteins such as Bax and Apaf-1, preventing the translocation of Bax to mitochondria and interactions of Apaf-1 with caspase-9 (31, 32).

Phosphorylation of substrate proteins by Aur-A often regulates their stability. Aur-A phosphorylates p53 and hepatoma up-regulated protein (HURP), leading to p53 degradation and HURP stabilization, respectively (27, 33). However, we did not observe any changes in the FAF1 protein level in association with its phosphorylation status. Instead, phosphorylation by Aur-A appears to endow “gain of function” to FAF1. Our preliminary analyses show that the introduction of FAF1 DD sensitizes cells to taxol (data not shown). Accordingly, we speculate that the phosphorylation event affords FAF1 an alternative function in addition to mediating the degradation of Aur-A. It is possible that phosphorylation of FAF1 allows or facilitates interactions with its partners which promote cell death.

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