Role for KAP1 Serine 824 Phosphorylation and Sumoylation/Desumoylation Switch in Regulating KAP1-mediated Transcriptional Repression*

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As a multifunctional protein, KAP1 (KRAB domain-associated protein 1) is reportedly subjected to multiple protein post-translational modifications, including phosphorylation and sumoylation. However, gaps exist in our knowledge of how KAP1 phosphorylation cross-talks with KAP1 sumoylation and what the biological consequence is. Here, we show that doxorubicin (Dox) treatment induces KAP1 phosphorylation at Ser-824 via an ataxia telangiectasia mutated (ATM)-dependent manner, correlating with the transcriptional de-repression of p21WAF1/CIP1 and Gadd45α. A S824A substitution of KAP1, which ablates the ATM-induced phosphorylation, results in an increase of KAP1 sumoylation and repression of p21 transcription in Dox-treated cells. By contrast, a S824D mutation of KAP1, which mimics constitutive phosphorylation of KAP1, leads to a decrease of KAP1 sumoylation and stimulation of p21 transcription before the exposure of Dox. We further provide evidence that SENP1 deSUMOylase is involved in activating basal, but not Dox-induced, KAP1 Ser-824 phosphorylation, rendering a stimulation of p21 and Gadd45α transcription. Moreover, KAP1 and differential sumoylation of KAP1 were also demonstrated to fine-tune the transcription of three additional KAP1-targeted genes, including Bax, Puma, and Noxa. Taken together, our results suggest a novel role for ATM that selectively stimulates KAP1 Ser-824 phosphorylation to repress its sumoylation, leading to the de-repression of expression of a subset of genes involved in promoting cell cycle control and apoptosis in response to genotoxic stresses.

The Krüppel-associated box zinc finger proteins (KRAB-ZFP) comprise approximately one-third of the 799 different zinc finger proteins, constituting the largest single-family transcriptional regulatory proteins in mammals (1). KRAB-domain-associated protein 1 (KAP1) functions as transcriptional corepressor for ZBRK1, a KRAB-ZFP member, by acting as a transcription intermediary factor to connect KRAB-ZFPs to transcriptional repression machinery. Because KAP1 itself cannot bind DNA directly, the specificity of transcriptional repression is dictated by its interaction with ZBRK1 through protein-protein interaction. The RING finger-B box-coiled-coil domain of KAP1 associates with the KRAB domain of ZBRK1, repressing the transcription of DNA damage-responsive gene Gadd45α (2) and p21WAF1/CIP1 (3). KAP1 can recruit and coordinate several components of gene silencing machinery. For example, KAP1 interacts with histone deacetylase complex NuRD and N-CoR1 and binds to histone methyltransferase SETDB1 to modify the configuration of chromatin structures (4–6). KAP1 also recruits heterochromatin protein 1 (HP1) to histones through a PXVXL motif (7, 8). In addition, KAP1 is identified as a Mdm2-binding protein that inactivates p53 (9, 10).

Emerging evidence supports the idea that post-translational modifications, including phosphorylation and sumoylation, play a pivotal role in regulating transcriptional control in response to different extracellular milieu. As a multifunctional protein, KAP1 is reportedly subjected to multiple post-translational modifications, and we have recently reported that sumoylation, a post-translational Lys modification, plays a major role in mediating KAP1 transcriptional co-repressor function and attenuating doxorubicin (Dox)-induced p21WAF1/CIP1 transcriptional activation in breast cancer MCF-7 cells (3). There are at least three Lys residues, 554, 779, and 804, that serve as the major sumoylation targets for KAP1, and the overall sumoylation capacity of KAP1 is transiently decreased upon Dox exposure. Moreover, the differential sumoylation status of KAP1 functions to modulate p21 transcription by switching the histone His-3–Lys-9 methylation and His-3–Lys-9 and -Lys-14 acetylation statuses without affecting the occupancy of the p21 proximal promoter by KAP1/ZBRK1 in MCF-7 cells. The KAP1 sumoylation-mimetic, SUMO-1–KAP1, desensitizes MCF-7 cells to Dox-induced cell death. Collectively, the KAP1 sumoylation/desumoylation switch suppresses KAP1 transcriptional

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co-repressor function by down-regulating histone His-3–Lys-9 methylation and fostering His-3–Lys-9 and -Lys-14 acetylation. However, it remains unclear what signal regulates the KAP1 sumoylation/desumoylation switch in response to Dox-induced DNA damage.

A major response to DNA damage insults, such as DNA double-strand breaks by x-ray irradiation or Dox treatment, is the activation of the nuclear protein kinase ataxia telangiectasia mutated (ATM) (11, 12). Patients with the genome instability syndrome ataxia telangiectasia are very sensitive to ionization radiation due to the lack of proper DNA damage response (11). Upon the occurrence of double-strand breaks, ATM is rapidly phosphorylated, and ATM’s own kinase activity subsequently phosphorylates a number of substrates, including H2AX, TopBP1, NBS1, and BRCA1 etc., resulting in the activation of cell cycle checkpoint control and DNA repair machinery (13). ATM belongs to a conserved protein family termed nuclear phosphatidylinositol 3-kinase-like kinases. Most members of the phosphatidylinositol 3-kinase-like kinase family possess serine/threonine kinase activity and a domain that is characteristic of phosphatidylinositol 3-kinase (14). Besides ATM, ataxia telangiectasia and Rad3-related (ATR), hSMG-1, mTOR, and the catalytic subunit of DNA-PK are the four other protein kinases of this family identified so far.

Numerous studies have established that the biological response to genotoxic stresses in mammalian cells is to trigger a complex network of transcriptional activation by the checkpointsignaling kinases ATM and ATR and their effector kinases Chk2 and Chk1, respectively (for review, see Ref. 15). One of the key consequences is cell cycle arrest and apoptosis, mediated by the induction of proteins involved in cell cycle control (p21WAF1/CIP1 and Gadd45α) and proapoptosis (Bax, Puma, and Noxa) (16–18). The balance of the combined induction of these genes leads to either cell cycle arrest or apoptosis when proapoptotic genes are transcribed at a level above a threshold, depending on the severity of DNA damage. Very recently, O’Geen at al. (19) have identified ~7000 KAP1 target sites by using chromatin immunoprecipitation assays coupled with a human 5-kilobase promoter array or a complete genomic tiling array. In light of the recent evidence for the multifunction of KAP1 (4–10, 19), it is tempting to speculate that the identified Dox-stimulated KAP1 desumoylation (3) could have a profound effect on the regulation of global gene expression in response to genotoxins. Moreover, KAP1 is demonstrated to be phosphorylated at Ser-824 by the phosphatidylinositol 3-kinase-like kinase family member of kinases after DNA damage.

Given that ATM regulates many aspects of DNA damage responses, including the induction of p21, we were intrigued by the possibility that ATM functions upstream of KAP1 SUMO-1 conjugation/deconjugation in response to DNA damage. Our results indicate that Dox treatment induces KAP1 Ser-824 phosphorylation and de-repression of Gadd45α and p21 transcription mainly via ATM, whereas the basal transcriptional co-repressor potential and sumoylation of KAP1 are coordinately regulated by SENP1 deSUMOylase. Finally, we demonstrate that the basal and DNA damage-induced transcription of a subset of KAP1-targeted genes, such as p21, Gadd45α, Bax, Puma, and Noxa, is coordinately regulated by the interplay of KAP1 sumoylation and phosphorylation. Our results could further unveil the role of a previously unnoticed ATM and KAP1 sumoylation switch in DNA damage responses.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 50 units/ml penicillin, and 50 μg/ml streptomycin, and MCF-7 cells were cultured in the same medium with an addition of 0.01 mg/ml recombinant human insulin in a humidified atmosphere of 37 °C and 5% CO2. The stable KAP1 knockdown cell line K928-c110 was grown as above with the addition of 10 μg/ml puromycin (8). Both ATM-deficient pEBS7 and ATM-proficient Y7S cells (22) were maintained in Eagle’s Dulbecco’s modified Eagle’s medium supplemented with 15% fetal bovine serum, antibiotics, 2 mM glutamine, 100 μg/ml hygromycin, and 1.25 units/ml nystatin in a humidified atmosphere of 37 °C and 5% CO2. G41 cells, U2OS (human osteosarcoma) stably transfected with a doxycycline-inducible ATR-kd (kinase-dead) construct (23), were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum plus 50 units/ml penicillin, 50 μg/ml streptomycin, 50 μg/ml hygromycin, and 200 μg/ml Geneticin.

**Western Analyses**—Whole cell lysates were prepared by lysing cells with radioimmunoprecipitation assay buffer (25 mM Tris, 125 mM NaCl, 1% Nonidet-P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide, pH 8.0) plus Complete Protease Inhibitor Mixture (Roche Applied Science) containing 10 mM N-ethylmaleimide, 1 mM NaF, and 2 mM Na2VO4 and subjected to SDS-PAGE followed by immunoblotting with antibodies for FLAG-KAP1 (M2, Sigma-Aldrich), tubulin (D-10) (Santa Cruz Biotechnologies), phospho-Ser-824-KAP1 (A300–767A, Bethyl Laboratories), and EGFP (Santa Cruz Biotechnologies). Blots were visualized with an enhanced chemiluminescence detection kit (ECL-Plus, Amersham Biosciences) and a Versadoc 5000 Imaging System (Bio-Rad). Densitometric data were obtained and analyzed with Quantity One Software (Bio-Rad). Results of Western analyses shown in this report are representative of two to four independent experiments.

**Luciferase Assays**—The p21-Luc reporter construct was made by subcloning a 2.3-kilobase p21 promoter into pGL3-Basic as previously described (3). The Gadd45α-Luc reporter was a gift from Dr. Wen-Hwa Lee at the University of California, Irvine. The luciferase reporters were co-transfected with a firefly control reporter, pRL-TK, for the purpose of normalization. Transfection was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instruction. Luciferase assays were carried out with DualGlo Luciferase assay kit (Promega), and the desired luciferase activity was calculated after normalization against the co-transfected firefly luciferase activity.
TABLE 1
Primer pairs used in the real-time RT-PCR experiments

| Primer    | Purpose  | Sequence (5’ to 3’) |
|-----------|----------|---------------------|
| 18 S rRNA FP | Real-time PCR | GUCGCAAGACCCCATGACAC |
| 18 S rRNA RP | RT and real-time PCR | GAAGTGAGGCAGGCCTGAGG |
| p21 FP | Real-time PCR | TTTCTTCTGGTCTCTCCGATG |
| p21 RP | RT and real-time PCR | GGCTTATATGCAAGCTGTTGGG |
| Gadd45α FP | Real-time PCR | AAGAAGTCTCTCCTGGCAGG |
| Gadd45α RP | RT and real-time PCR | GGCAACAACCCACATTCACTGCDG |
| Bax FP | Real-time PCR | CCGAATCTTACCCTCTCCGATG |
| Bax RP | RT and real-time PCR | CAAATCCAGGAGCTGAGG |
| Noxa FP | Real-time PCR | ATATCCGCTGGCCTGCTG |
| Noxa RP | RT and real-time PCR | GGTCGAGATTGCAGCACTGAA |
| Puma FP | Real-time PCR | CGTGAATCCCTGGTCTG |
| Puma RP | RT and real-time PCR | ATAGATGCCGAGCTGTCACA |

In Vivo Sumoylation Assays—The in vivo sumoylation assay was carried out with co-transfection of FLAG-KAP1 or its mutants and EGFP-SUMO-1 in a 1:4 ratio into HEK293 cells with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instruction. SUMOylated KAP1 was detected by immunoblotting of whole cell lysates against FLAG or EGFP tag.

Immunoprecipitation of KAP1—Transfection of FLAG-KAP1 or FLAG-KAP1 with EGFP-SUMO-1 expression constructs into HEK293 or MCF-7 cells was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s manual. Whole cell lysates were prepared by lysing cells with lysis buffer (50 mM Tris-HCl, pH 6.8, 100 mM NaCl, 0.5 mM MgCl2, 1 mM EDTA, 0.2% Nonidet P-40, 1 mM Dithiothreitol, 1× protease inhibitor mixture (Roche Applied Science), 10 mM N-ethylmaleimide (Sigma-Aldrich). For each sample, 5 μl of anti-FLAG M2 antibody was mixed with 1 μg of whole cell lysates and incubated on ice for 2 h. Then Protein A/G PLUS-agarose (Santa Cruz Biotechnology, CA) was added, and the sample was rotated at 4°C overnight. The mixture was then washed with 1 ml of 1× phosphate-buffered saline three times. Immunoprecipitates were then eluted in 40 μl of 2× SDS sample buffer, and half of the elution was subjected to immunoblotting analyses.

Total RNA Extraction, Reverse Transcription, and Real-time PCR—Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. The total RNA from Dox-treated and control MCF-7 cells was then treated with RNase-free DNase (Invitrogen) and extracted again with phenol-chloroform followed by ethanol precipitation. Reverse transcription and quantitative PCR of p21, Gadd45α, Bax, Puma, and Noxa mRNA were performed with iTaq SYBR Green Supermix (Bio-Rad), a fraction of each total RNA sample, and specific pairs of gene-specific primers (Table 1). PCR amplification and fluorescence detection were done with MyIq real-time PCR detection system, and the threshold cycles were determined by iCycler program with its default setting. Fold inductions were determined by the ΔΔCt method against 18 S rRNA.

Constructs—Human SENP1 cDNA was amplified from testis cDNA library (Clontech) and cloned into EcoRI and EcoRV sites of pCMV-HA vector, yielding hemagglutinin-SENP1. Hemagglutinin (HA)-SENP1C603S mutant was constructed from HA-SENP1 plasmid by QuikChange site-directed mutagenesis kit (Stratagene). Oligonucleotides corresponding to SENP1 nucleotide sequence 413 ACCCATCACTGCATGGATCC491 and 538 ACTCGAGGCGCAGCATGTTA258 were inserted into pSuper vector (Oligoengine) to generate sh-SENP1-1 and sh-SENP1-2, respectively. FLAG-KAP1(S824D), FLAG-KAP1(S824A), and SUMO-1-KAP1(L306P) mutants were engineered using FLAG-KAP1 and SUMO-1-KAP1 (3) as a template, respectively, by a QuikChange site-directed mutagenesis kit (Stratagene).

Inducible sh-KAP1—Tet-inducible sh-KAP1 was constructed according to van de Wetering et al. (24). The oligonucleotides were used as follows: for KAP1, 5′-GATCCCTTGGAGCCCTATGG-3′ and 5′-AGCTTTTCCAAAATGGAGCCCATGGGCGCCACGTCCTGTCAG-3′; for the control, 5′-GATCCCTCTTATATGCAAGCTGTTGGG-3′. The oligonucleotides mixture and 100 ng of purified pTERR+ backbone were used per ligation. Clones expressing pTERR+shKAP1 were selected with ampicillin resistance. To test the effect of sh-KAP1 knockdown, MCF-7 cells were co-transfected with p21-Luc, Tet-repressor plasmid pCDNA6/TR (Invitrogen), and pTER-H11001 was cut with BglII and HindIII, and the pTER-H11001 backbone was purified from the gel. Sense and antisense oligos (100 pmol of each) were annealed in 50 μl of annealing buffer (10 mM potassium acetate, 3 μM Hepes-KOH, pH 7.4, and 0.2 mM magnesium acetate). One microliter of this oligos mixture and 100 ng of purified pTERR+ backbone were used per ligation. Colonies expressing pTERR+shKAP1 or pTERR+sh-Control were selected at 24 h post-transfection, cells were treated with 2 μM doxycycline for 4 or 24 h to induce the expression of their respective short hairpin RNA. Then the luciferase activity was measured and normalized against pRL-TK. pCDNA6-TO-Luc (Invitrogen) was used as positive control for doxycycline induction.

Statistical Analysis—The error bar represents the S.D. of the mean. Statistical analyses were performed using one-way analysis of variance followed by post hoc comparisons based on a modified Newman-Keuls-Student procedure with p < 0.05 considered significant. Where appropriate, unpaired Student’s t tests were also performed to determine the difference between two data groups.

RESULTS

Differential Activation of ATM and ATR in Response to Dox and UV Treatment—We have recently demonstrated that the transcription of p21WAF1/CIP1 is distinctly regulated by KAP1 sumoylation status; sumoylation-mimetic KAP1 (SUMO-1-KAP1, Fig. 1A, left panel) enhances transcriptional repression by increasing the methylation of histone His-3–Lys-9 and decreasing the acetylation of His-3–Lys-9 and -Lys-14, whereas sumoylation-defective KAP1 (KAP1(S3K/R), Fig. 1A, left panel) relieves transcriptional repression in an opposite manner (3). Here, we report that Dox-treatment also activated the transcription of the DNA damage response gene Gadd45α (growth arrest and DNA damage clone 45) in a way similar to that of p21, whereas transfected KAP1 repressed the Dox-induced Gadd45α transcription in MCF-7 cells (Fig. 1A, right panel,
ATM Coordinates KAP1 Phosphorylation and Sumoylation

A

B

C

D

E

Gadd45α mRNA

Fold Induction by Dox

KAP1
S-KAP1
S-KAP1-L306P

Y5 cells (ATM-proficient)

Relative Induction of Gadd45a-Luc

-UV +UV -Dox +Dox

KAP1
SUMO-1-KAP1 (l)
SUMO-1-KAP1 (h)
SUMO-1-KAP1(L306)

pEBS7 cells (ATM-deficient)

Relative Induction of Gadd45a-Luc

-UV +UV -Dox +Dox

KAP1
SUMO-1-KAP1 (l)
SUMO-1-KAP1 (h)
SUMO-1-KAP1(L306)

GK41 cells (Tet-On ATR-kd)

Relative Induction of Gadd45a-Luc

Doxycycline

Dox

- UV +UV -Dox +Dox
second lane 2 versus first lane). Consistent with a previous report (3), sumoylation-mimetic SUMO-1-KAP1 abolished the ability of Dox to induce Gadd45α expression (Fig. 1A, right panel, third lane). Notably, a L306P mutation of SUMO-1-KAP1 (SUMO-1-KAP1(L306P); Fig. 1A, left panel), which disrupts the interaction between coil-coiled domain of SUMO-1-KAP1 and KRAB domain with ZBRK1, almost completely relieved the repressive effect by SUMO-1-KAP1 (Fig. 1A, right panel, second lane). Our results support the notion that sumoylation of KAP1 enhances KAP1’s function as a transcriptional co-repressor to attenuate Gadd45α transcription.

Because both ATM and ATR function as both the sensors for and activators of DNA break repair, we set to examine whether ATM, ATR, or both are involved in mediating Dox-induced relief of KAP1 transcriptional co-repressor function. ATM-deficient fibroblast cells (pEB7) and their counterparts (YZ5, in which the expression of wild-type ATM is restored by stable transfection of ATM in pEB7 cells) were employed to explore the mechanism underlying KAP1/ZBRK1-mediated gene expression regulation in response to Dox treatment. As shown in Fig. 1B, ATM was rapidly and transiently phosphorylated on Ser-1981 after Dox treatment in MCF-7 cells with comparable kinetics to that observed in ATM-proficient YZ5 cells. The ATM-deficient pEB7 cells served as a control to ensure the validity of the antibody against Ser-1981-phosphorylated ATM (Fig. 1B).

We next compared the inducibility of Gadd45α-Luc in response to UV and Dox treatment in pEB7 and YZ5 cells. In ATM-deficient pEB7 cells, the Gadd45α transcription was induced by UV radiation (Fig. 1C, lanes 5–7), consistent with the idea that UV-induced DNA damage responses are mainly not ATM-dependent. Notably, Dox-induced Gadd45α transcription in pEB7 cells was modest (Fig. 1C, lane 13), implying that ATM is required for Dox-mediated transcriptional activation. By contrast, Dox treatment induced Gadd45α-Luc activation (Fig. 1D, lane 13 versus lane 5), which was compromised by the expression of SUMO-1-KAP1 in a dose-dependent manner (Fig. 1D, lanes 14 and 15 versus lane 13) in ATM-proficient YZ5 cells. Although UV radiation activated a comparable induction in Gadd45α transcription (Fig. 1D, lanes 1 and 5 versus lanes 9 and 13), SUMO-1-KAP1 conferred a negligible effect on UV-induced Gadd45α promoter activation in YZ5 cells (Fig. 1D, lanes 6 and 7). Collectively, our results suggest that Dox and UV radiation induce Gadd45α transcription in distinct manners, and the sumoylation-mimetic SUMO-1-KAP1 only represses ATM-mediated Gadd45α transactivation.

GK41 cells, human osteosarcoma U2OS cells stably transfected with doxycycline-inducible ATR-kd, were used to assess the role of ATR in KAP1-dependent Gadd45α transcriptional de-repression by Dox exposure. As shown in Fig. 1E, Dox treatment induced Gadd45α transcription by 2.4-fold (lane 4 versus lane 1), and the expression of SUMO-1-KAP1 repressed both basal (lanes 2 and 3) and Dox-inducible Gadd45α transcription (lanes 5 and 6) in the absence of doxycycline (such that the expression of ATR-kd is negligible) in GK41 cells. The expression of ATR-kd (by adding doxycycline (1 μg/ml) to medium) did not affect the basal and Dox-inducible Gadd45α transcription under the same experimental protocol (Fig. 1E, lanes 7 and 10–12 versus lanes 1 and 4–6). Taken together, we conclude that Dox treatment induces Gadd45α transcriptional activation via an ATM-dependent pathway to relieve KAP1-mediated trans-repression. However, it appears that SUMO-1-KAP1 had a lesser effect in repressing Dox-induced Gadd45α transcription in GK41 cells than in YZ5 cells. The exact mechanism underlying this discrepancy remains to be elucidated.

**Dox Treatment Induces KAP1 Phosphorylation at Ser-824 via ATM**—To further examine the role of KAP1 Ser-824 phosphorylation in governing KAP1 function, we next engineered KAP1 mutants containing S824A or S824D substitutions. The S824D mutant was expected to mimic Ser-824-phosphorylated KAP1, whereas the S824A mutant reflected non-phosphorylated KAP1. By using KAP1(wt)–, KAP1(S/A)–, or KAP1(S/D)–transiently transfected MCF-7 cells, we then confirmed the specificity of antibody for Dox-induced KAP1 Ser-824 phosphorylation (Fig. 2A, left panel, lanes 4 and 7). The anti-KAP1 Ser-824 antibody has been used by Ziv et al. (21) to demonstrate that ATM phosphorylates KAP1 at Ser-824 in response to double-strand breaks. By using the level of tubulin to normalize for equal loading and the level of KAP1 to normalize for transcription efficiency, the quantitative analysis of Dox-induced KAP1 Ser-824 phosphorylation in MCF-7 cells was summarized in Fig. 2A (right panel). The phospho-Ser-824 signals detected in KAP1(S/A)- and KAP1(S/D)-transfected cells after treatment with Dox (1 μM) for 3 h represented the Ser-824-phosphorylated endogenous KAP1 (Fig. 2A, left panel, lanes 8 and 9).

Our previous data suggest that ATM may be the key phosphatidylinositol 3-kinase protein-like kinase that induces Gadd45α transcriptional de-repression in Dox-treated cells, as Dox-induced Gadd45α-Luc activity was significantly reduced in ATM-deficient pEB7 cells (Fig. 1C) as compared with that of ATM-complemented YZ5 cells (Fig. 1D). The possible role of ATM in mediating Dox-induced KAP1 Ser-824 phosphorylation...
ATM Coordinates KAP1 Phosphorylation and Sumoylation

**A**

![Western blot images](image)

**B**

![Western blot images](image)

**C**

![Graphs](image)

FIGURE 2. A, DOX treatment induces KAP1 phosphorylation on Ser-824. MCF-7 cells were transiently transfected with vector, KAP1(wt), KAP1(S824A), and KAP1(S824D). Twenty-four h after transfection cells were treated with DOX (5 μM) for 30 min or DOX (1 μM) for 3 h. Western analyses on total cellular proteins with indicated antibodies were performed. One representative Western blot from three independent experiments is shown (left panel). The corresponding quantitative analyses on the relative KAP1 Ser-824 phosphorylation levels in individual sample after normalizing with tubulin level are shown (right panel). B, ATM is required for DOX-induced KAP1 phosphorylation at Ser-824. pEBS7 and YZ5 cells were treated with DOX (1 μM) and harvested 3 h later. Total cellular extract underwent Western analyses with the indicated antibodies (left panel). The corresponding quantitative analyses on the relative KAP1 Ser-824 phosphorylation levels in lanes 5–8 shown in the left panel after normalizing with endogenous KAP1 Ser-824 phosphorylation levels (lanes 1–4) and tubulin levels for equal loading are shown (right panel). C, KAP1 Ser-824 phosphorylation is essential for de-repressing its transcriptional co-repressor activity. MCF-7 cells were individually transfected with p21-Luc and a combination of ZBRK1 and wild-type KAP1 or its mutants. Twenty-four h after transfections cells were treated with DOX (1 μM) for 3 h, and the luciferase activity was measured. Each bar represents the mean ± S.D. from three independent experiments.

To investigate whether KAP1 sumoylation conversely inhibits its Ser-824 phosphorylation in the absence or presence of DOX treatment, we performed immunoprecipitation followed by Western analysis to probe both basal and DOX-induced KAP1 Ser-824 phosphorylation levels in cells with or without exogenous SUMO-1. As shown in Fig. 3B, KAP1 is SUMOylated by exogenous SUMO-1 (left panel, lane 2). The quantitatively analysis (Fig. 3B, right panel) confirmed that there was no KAP1 species with both SUMOylated and phosphorylated exclusively from ATM activation.

To investigate the relationship between KAP1 Ser-824 phosphorylation status and its transcriptional co-repressor activity, transient transfection assays of p21-Luc activation with individual wild-type KAP1 or its engineered mutants into MCF-7 cells were performed. As shown in Fig. 2C, whereas KAP1(S/A) suppressed DOX-induced p21 promoter activation, the mutation of S824D in KAP1 greatly enhanced the un-stimulated p21 promoter activity.

To illustrate the possible relationship between KAP1 Ser-824 phosphorylation and sumoylation, in vivo sumoylation assays were carried out by co-transfecting wild-type KAP1 or its mutants and SUMO-1 in HEK293 cells in the presence or absence of DOX treatment and followed by Western analyses. Consistent with a previous report (3), KAP1(wt) sumoylation decreased by about 40% upon exposure to DOX (Fig. 3A, upper panel, lane 6 versus lane 2). By contrast, KAP1(S824A) sumoylation levels, irrespective of treatment with vehicle or DOX, remained at almost the same level as that of KAP1(wt) detected in vehicle-treated cells (Fig. 3A, upper panel, lanes 7 and 3 versus lane 2). Moreover, KAP1(S824D) exhibited a marked decrease of its sumoylation levels in both vehicle- and DOX-treated cells (Fig. 3A, upper panel, lanes 4 and 8 versus lane 2). After normalization with the level of tubulin for equal loading and of KAP1 for transfection efficiency, the relative % sumoylation of KAP1 and its mutants suggested that Ser-824 phosphorylation plays a pivotal role in affecting KAP1 sumoylation status (Fig. 3A, lower panel).
ATM Coordinates KAP1 Phosphorylation and Sumoylation

![Diagram](image)

**FIGURE 3.** A, SB24A mutation represses KAP1 sumoylation. MCF-7 cells were transfected with FLAG-tagged wild-type (WT) KAP1 or its mutants in the presence or absence of EGFP-SUMO-1 under treatment with vehicle or Dox (1 μM) for 3 h. Protein extracts were analyzed with an anti-FLAG, anti-EGFP, anti-Ser(P)-824-KAP1 (pS824-KAP1), or anti-tubulin antibody by western analyses. One representative Western blot from three independent experiments is shown (left panel). The corresponding quantitative analyses on the relative % of KAP1 sumoylation in each individual sample after normalizing with tubulin level and transfection efficiency are shown (right panel). The asterisk denotes the signals detected in vehicle- or Dox-treated cells (Fig. 3B, left panel, lanes 5 and 6). Last, cells were transfected with either KAP1(wt), sumoylation-defective KAP1(3K/R), or sumoylation-mimetic SUMO-1-KAP1 and subjected to vehicle or Dox treatment to probe their respective phosphorylation profiles. Consistent with Fig. 3B, SUMO-1-KAP1 lacked a Ser(P)-824 (pS824)-SUMO-1-KAP1 signal comparable with that observed for KAP1(wt) upon Dox exposure (Fig. 3C, left panel, lane 6, indicated by an asterisk). Quantitative analyses further confirmed that KAP1(3K/R), in the absence of Dox, exhibited an almost 2-fold Ser-824 phosphorylation signal compared with KAP1(wt) and that Dox treatment failed to further induce KAP1(3K/R) Ser-824 phosphorylation (Fig. 3C, right panel, lanes 2 and 5 versus lanes 1 and 4). Although the basal KAP1 Ser-824 phosphorylation detected in anti-FLAG-immunoprecipitates (Fig. 3B, lane 3) may reflect endogenous ATM activation, we noticed that exogenous SUMO-1 reproducibly enhances basal KAP1 Ser-824 phosphorylation level by about 70% (Fig. 3B, lane 4 versus lane 3). The exact reason underlying this stimulation by enhanced global sumoylation is still unclear.

**SENP1 Enhances Basal Ser-824 Phosphorylation of KAP1**—To test whether SUMO-specific protease regulates the ability of KAP1 to repress p21 transcription, SENP1 and SENP2 were each co-transfected with p21-Luc and a combination of KAP1 and ZBRK1 in MCF-7 cells. As shown in Fig. 4A, p21-Luc activity was induced upon Dox treatment in MCF-7 cells transfected with wild-type ZBRK1 and KAP1 (lane 2 versus lane 1), whereas p21 transcription was held nearly unchanged by Dox treatment in SUMO-1-KAP1-transfected cells (lane 4). Yet MCF-7 cells transfected with SENP1 displayed a 3.6-fold increase in the basal p21 transcription (Fig. 4A, lane 5 versus lane 1). Importantly, the co-transfection of uncleavable SUMO-1-KAP1 with SENP1 suppressed the SENP1-mediated stimulation (Fig. 4A, lane 6 versus lane 5), suggesting that the potential desumoylation of KAP1 by SENP1 could have accounted for the increased basal p21 transcription. By contrast, SENP2 conferred almost no...
FIGURE 4. A, SENP1 activates p21 transcription. MCF-7 cells were transfected with p21-Luc reporter along with a combination of ZBRK1, KAP1, SUMO-1, KAP1, SENP1, and SENP2, as indicated. At 40 h post-transfection, the luciferase activity was measured and normalized against a firefly control reporter (pRL-TK). Results represent the mean ± S.D. from three independent experiments. B, SENP1 deSUMOylates SUMO-modified KAP1. HEK293T cells were transfected with KAP1, EGFP-SUMO-1, and SENP1 or SENP1C603S (SENP1cs). SUMO conjugation of KAP1 was analyzed by Western analyses using an anti-FLAG antibody. C, SENP1 induces basal KAP1 Ser-824 phosphorylation. Lysates of cells transfected with KAP1 and SENP1 or SENP1cs followed by vehicle or Dox (1 μM) treatment for 3 h were analyzed with anti-FLAG, anti-Ser(P)-824-KAP1, and anti-tubulin antibodies, respectively. s: short exposure and l: long exposure. D, Dox-induced Ser-824 phosphorylation of KAP1 is SENP1-independent. Cells were transfected with pSUPER empty vector, sh-SENP1-1, or sh-SENP1-2 with KAP1 and followed by treatment with vehicle or Dox (1 μM) for 3 h. The KAP1 Ser-824 phosphorylation level was measured with an anti-Ser(P)-824-KAP1 antibody. One representative Western blot from three independent experiments is shown. s: short exposure; l: long exposure. E, knock down of SENP1 inhibits p21 and Gadd45α expression. MCF7 cells were transfected with pSUPER empty vector, sh-SENP1-1 or sh-SENP1-2 with KAP1, p21-Luc or Gadd45α-Luc. At 40 h post-transfection the luciferase activity was measured and normalized against a firefly control reporter (pRL-TK). * denotes p < 0.10; ** denotes p < 0.05.
effect on the p21 transcription in the absence of Dox (Fig. 4A, lanes 7 and 8), further implicating the specificity of SENP1 in regulating KAP1 function in the absence of Dox.

To determine whether SENP1 action is mediated through deSUMOylating KAP1, 293T cells were co-transfected with KAP1, SUMO-1, and either SENP1 or SENP1C603S, and the profile of SUMO-1-modified KAP1 was determined. Co-transfection with wild-type SENP1 led to a reduction in the level of SUMOylated KAP1 (Fig. 4B, lane 6 versus lane 4). As expected, the enzymatic activity of SENP1 was required for deSUMOylation of KAP1 since the SENP1C603S mutant rendered an accumulation of high molecular weight SUMOylated KAP1 (Fig. 4B, lane 7 versus lane 4). We next determined whether SENP1 could directly induce phosphorylation of KAP1 at Ser-824. As shown in Fig. 4C, co-expression of SENP1 induced basal KAP1 Ser-824 phosphorylation (lane 2 versus lane 1), whereas C603S mutation of SENP1 attenuated such a function of SENP1 (lane 3 versus lane 2). However, neither SENP1 nor SENP1C603S elicited any stimulating or inhibitory effect on KAP1 Ser-824 phosphorylation in Dox-treated cells (Fig. 4C, lanes 5 and 6). Based on these results, we suggest that SENP1 only fine-tunes the basal, but not Dox-modulated, KAP1 co-repressor activity by regulating its Ser-824 phosphorylation status. We then wished to confirm the possible link between SENP1 and KAP1 function by using short hairpin RNA to knock down endogenous SENP1 levels. Although expression of sh-SENP1-1 or sh-SENP1-2 reduced SENP1 levels. Although expression of sh-SENP1-1 or sh-SENP1-2 elicited a doxycycline-induced sh-KAP1, as shown in Fig. 4B, left panel, knockdown of KAP1 clearly induced the basal expression of p21 and Noxa. Furthermore, although Dox treatment had almost no effect on the expression of Bax, Gadd45α, Puma, and Noxa in HEK293 cells, KAP1 knock-down allowed the Dox to induce the expression of Bax, Gadd45α, Puma, and Noxa as well as p21. Next, we attempted to transiently deplete KAP1 in MCF-7 cells by using a doxycycline-inducible sh-KAP1. As shown in Fig. 5B (right panel), a time-dependent induction of p21-Luc by sh-KAP1, but not by sh-control, was observed, whereas a doxycycline-induced pCDNA4TO-Luc served as a control (data not shown). The lack of robust induction of basal p21-Luc activity in MCF-7 cells transfected with sh-KAP1 compared with the increase of basal p21 mRNA level by sh-KAP1 in HEK293 cells may reflect the low KAP1 knockdown efficiency by transient transfection in MCF-7 cells and/or additional DNA elements involved in the regulation of p21 expression.

Last, to examine the role of differential sumoylation of KAP1 in regulating the expression of p21, Gadd45α, Bax, Puma, and Noxa in response to DNA damage, MCF-7 cells were transfected with either wild-type, sumoylation-mimetic, or sumoylation-defective KAP1 followed by treatment with Dox (1 μM) for 4 h. As shown in Fig. 5C, the steady-state levels of these five mRNAs exhibited a general concordance of up-regulation at 4 h post-treatment; 4.3-fold for p21, 3.0-fold for Gadd45α, 1.6-fold for Bax, 2.3-fold for Puma, and 2.1-fold for Noxa. The introduction of SUMO-1-KAP1 suppressed the induction of all these five mRNAs, whereas the expression of KAP1(3K/R) enhanced the mRNA levels of all these five genes, although the extent of enhancement by KAP1(3K/R) was more modest than the magnitude of suppression by SUMO-1-KAP1 (Fig. 5C). Together with Fig. 5B, the up-regulation in the expression of these five examined genes by sh-KAP1 in the context of DNA damage was more prominent in p21 than in Gadd45α, Bax, Puma, and Noxa. Collectively, we conclude that both KAP1 and the KAP1 sumoylation switch play critical roles in regulating the expression of these five ZBRK1-binding element-harboring genes, conceivably contributing to the DNA damage response.

**DISCUSSION**

ATM is one of major kinases involved in activating DNA damage responses, which span many signaling pathways including the cell cycle checkpoints (12). Here, a novel ATM-KAP1 signaling pathway linking genome surveillance to a key transcriptional co-repressor to dynamically regulate the expression of a subset of genes involved in promoting cell cycle arrest and proapoptosis is demonstrated. We present evidence for the involvement of ATM in transcriptional de-repression of Gadd45α (Fig. 1) and a correlation between KAP1 Ser-824 phosphorylation, KAP1 desumoylation, and a down-regulation of KAP1 transcriptional co-repressor function (Figs. 2 and 3). Furthermore, SENP1-mediated KAP1 desumoylation is critical for the basal activation of Gadd45α and p21 by facilitating Ser-
FIGURE 5. A, the p53-responsive element and putative ZBRK1 binding elements (BE) at the promoters of Bax, Puma, and Noxa genes. The positions of p53-responsive elements and the ZBRK1 binding elements, according to previous reports or searched against the 15-bp consensus motif GGGXXXCAGXXXTTT with the permission of mismatches less than two nucleotides by ScanProsite program, are denoted. The transcription start site of each gene is defined as +1 position, and the corresponding ATG translation initiator is also shown. The two ZBRK1 binding elements found in Noxa partially overlap. B, sh-KAP1 de-represses the ZBRK1-mediated inhibition of p21, Gadd45α, Bax, Puma, and Noxa expression. Quantitative real-time RT-PCR analyses of p21, Gadd45α, Bax, Puma, and Noxa mRNA levels in HEK293 and stable KAP1 knockdown cell line K928-cI10 (left panel) are shown. Cells were treated with Dox (1 μM) for 3 h, and the total RNAs were extracted by TRIzol reagent. The respective p21, Gadd45α, Bax, Puma, and Noxa mRNA levels were then quantitated by one-step reverse transcription and real-time PCR with gene-specific primers (Table 1). The relative mRNA level for each gene in a different context was calculated by \( \Delta \Delta C_t \) method against 18 S rRNA, and the level of each gene in vehicle-treated HEK293 cells was arbitrarily assigned as 1. Each bar represents the mean ± S.D. from three independent experiments. MCF-7 cells were co-transfected with p21-Luc, Tet-repressor plasmid pCDNA6/TR and pTER \(^{\text{Tet}}\)-sh-KAP1 or pTER \(^{\text{Tet}}\)-Control (right panel). At 24 h post-transfection, transfected cells were treated with 2 μM doxycycline for 4 or 24 h. Then the luciferase activity was measured and normalized against pRL-TK. C, SUMO-1-KAP1 represses Dox-induced p21, Gadd45α, Bax, Puma, and Noxa expression in MCF-7 cells. MCF-7 cells transiently transfected with wild-type KAP1, SUMO-1-KAP1, or KAP1(3K/R) were treated with Dox (1 μM) for 4 h. The total RNA isolation and quantitative RT-PCR analyses were performed as described in B. The -fold induction of each gene in response to Dox treatment was calculated by \( \Delta \Delta C_t \) method against 18 S rRNA. The results represent the mean ± S.D. from three independent experiments.
ATM Coordinates KAP1 Phosphorylation and Sumoylation

824 phosphorylation (Fig. 4). Our results further demonstrate that Ala substitution at Ser-824 (KAP1(S/A)) and Asp substitution at Ser-824 (KAP1(S/D)) gave opposite profiles of sumoylation and stimulation of p21 basal expression (Figs. 2 and 3) and that SUMO-1-KAP1 resisted, at least in part, Dox-elicited Ser-824 phosphorylation (Fig. 3C). We postulate that Dox-mediated ATM activation plays a critical role in the inhibition of KAP1 sumoylation and the subsequent de-repression of five ZBRK1-binding element-harboring, cell cycle control and proapoptotic genes, including p21 and Gadd45α, Bax, Puma, and Noxa.

To our knowledge the current study reported herein is the first one to suggest the inhibition of KAP1 sumoylation and its trans-repression function by ATM-dependent KAP1 Ser-824 phosphorylation. As shown recently by Ziv et al. (21) and White et al. (20), KAP1 is phosphorylated at Ser-824 after DNA damage. Interestingly, DNA damage-induced KAP1 phosphorylation results in a dynamic co-localization of pSer-824-KAP1 with numerous damage response factors at DNA lesions and chromatin decondensation (20, 21). Notably, Ziv et al. (21) reported that no major changes were detected in the interactions between KAP1 and HP-1, SETDB1, and Mi-2α proteins after the induction of DNA damage, suggesting the possibility that SUMOylated KAP1, not KAP1 per se, plays a functional role in modulating KAP1-dependent chromatin condensation and subsequent repression of gene expression. Conceivably, the reported KAP1 Ser-824 phosphorylation-mediated inhibition of KAP1 sumoylation could play an essential role in fine-tuning the biological function of KAP1.

The fold-of-activation on Gadd45α transcription by UV irradiation and Dox appeared to be comparable in ATM-proficient YZ5 and ATM-deficient pEB87 cells. However, SUMO-1-KAP1 was able to repress Dox-induced, but not UV-elicited, Gadd45α transcription, indicating that the mechanisms underlying transcriptional induction by Dox and UV are distinct. It was previously shown that ZBRK1 was subjected to polyubiquitination and proteasome-dependent degradation upon UV irradiation (27), and therefore, the exogenous expression of SUMO-1-KAP1 would exert no inhibitory effect on UV-induced Gadd45α transcription. Although it has been generally accepted that ATM and ATR exhibit some redundancy in activating Chk1 and Chk2, a very modest, if any, reduction in the Dox-induced Gadd45α transcription was observed in the presence of ATR-kd. Hence, we conclude that ATM–KAP1 signaling is mainly specific for de-repression of ZBRK1-binding element-containing genes in response to Dox and perhaps other agents causing DNA double-stranded breaks. The observation that only about 30% of identified KAP1 target promoters were also occupied by repressive methylated His-3–Lys-9 or -K27 (19) further supported our notion that the dynamic Ser-824 phosphorylation-mediated sumoylation/desumoylation switch could affect KAP1 transcriptional co-repressor function.

Knowledge about protein sumoylation/desumoylation has increased enormously in the past few years. However, the mechanism regulating SUMO conjugation or de-conjugation is far from understood. Various stresses, including heat-shock, hypoxia, osmotic shock, and oxidative stress, are known to regulate the global SUMO conjugation pathway (for review, see Refs. 28–30). One obvious means to regulate sumoylation processes is to modulate the phosphorylation status of targets. For example, phosphorylation on Ser-303 of HSF1 is demonstrated to enhance sumoylation (31). Notably, this finding has been extended to many transcriptional regulators, such as GATA-1 and MEF2 (32, 33). By contrast, sumoylation of c-Jun and c-fos is negatively regulated by their respective phosphorylation via JNK (for c-Jun) and an unidentified, Ras-activated kinase (for c-fos) (34, 35). Moreover, phosphorylation is also shown to inhibit the sumoylation of p53 and Elk-1 (36, 37). Meanwhile, because protein sumoylation is a dynamic and transient process, our studies have been restricted to use of sumoylation-mimic or sumoylation-defective mutants to study the effect by sumoylation on KAP1 Ser-824 phosphorylation. Whether Ser-824 phosphorylation serves to recruit deSUMOylase, block access of Ubc9 or E3 ligases, or prevent sumoylation through some other mechanism is currently under investigation.

We demonstrate herein that SENP1, not SENP2, is able to mimic Dox-induced down-regulation of KAP1 sumoylation, thus activating basal p21 transcription at a magnitude comparable with that of Dox treatment. SENP1 is presumably a nuclear SUMO-specific protease based on the observations that transient expression of SENP1 disrupts promyelocytic leukemia (PML) oncopgenic domains (PODs), a subnuclear structure of SUMOylated PML, without affecting the level of SUMOylated RanGAP-1, which resides in the cytoplasmic periphery of nuclear pore complex (38). Additionally, SENP1 reportedly participates in androgen receptor-mediated signaling and c-Jun-dependent transcription (39, 40). It has been speculated that the observed transcriptional activation by SENP1 is mediated by the desumoylation of androgen receptor/c-Jun-interacting partners, such as p300 and HDAC1 (39, 40), or of KAP1 (this report), thus relieving the repressive ability endowed by SUMO conjugation. Last, our findings that SENP1 alone is sufficient to induce basal p21 expression is also consistent with our previous report that sumoylation-defective KAP1(3K/R) increases basal p21 transcription (3), suggesting that ATM preferentially phosphorylates unSUMOylated KAP1.

As shown in Fig. 5, there are a number of other genes involved in the control of cell-cycle progression, DNA damage repair, and apoptosis whose expressions are also subjected to the repression by KAP1. It is reasonable to assume that differential KAP1 sumoylation regulates the transcription of these five genes as p21, Gadd45α, Bax, Puma, and Noxa before or after the onset of DNA damage. Presumably, the proposed mechanism by which ATM, in collaboration with KAP1 sumoylation switch, modulates the transcription of these five genes. Although the p53-mediated transcriptional activation of cell cycle arrest and proapoptotic genes has been well documented (18, 41, 42), p53-null or -compromised cells are still sensitive to genotoxic stress (43, 44). In fact, Dox treatment induces p21 expression, albeit at lower level, in p53−/− or -compromised cells (Ref. 45 and this report), underscoring the possibility of regulating cell cycle arrest and apoptosis by both p53-dependent and p53-independent pathways in response to genotoxic stresses. Although the role of KAP1 in repressing the expression of these five examined genes is less universal in HEK293
cells, we propose that KAP1 phosphorylation/sumoylation switch or sh-KAP1 could complement p53 in activating the expression of a subset of DNA damage target genes. Current efforts are aimed at determining whether different cofactors or influences are necessary for KAP1 to repress the expression of target genes in different p53 contexts.

Based on the data presented herein, we propose the following model for the role of sumoylation and phosphorylation in regulating KAP1 co-repressor activity; KAP1 exists in a balance between SUMOylated KAP1 (active co-repressor) and Ser-824-phosphorylated KAP1 (inactive co-repressor) via ATM activation from endogenous double-strand breaks during processes such as meiosis and DNA replication (46). By doing so KAP1 is able to set a basal transcription level for a subset of KAP1-targeted cell cycle checkpoint genes and responds timely to exogenous genotoxic stresses. After Dox treatment, the robust ATM activation induces marked KAP1 Ser-824 phosphorylation, in turn, de-repressing the transcription of p21, Gadd45α, Bax, Puma, and Noxa. In our model we further predict that SENP1 sets a threshold for basal KAP1 sumoylation and facilitates KAP1 Ser-824 phosphorylation. It is imperative to note that our proposed model is simplistic, and we only provide evidence that SUMO-1-KAP1 partially resists Dox-induced Ser-824 phosphorylation and KAP1(S/D) fails in part to be SUMOylated. The molecular mechanisms underlying the Dox-induced KAP1 Ser-824 phosphorylation-mediated inhibition of sumoylation and the failure of ATM to efficiently phosphorylate SUMOylated KAP1 remain to be established. Almost certainly, the regulation of KAP1 Ser-824 phosphorylation involves another kinase(s) and phosphatase(s) to provide a dynamic, temporally selective regulation of the KAP1 Ser-824 phosphorylation/sumoylation switch. Likewise, the same Lys residue(s), the target(s) of sumoylation, could be subjected to other post-translational modifications, such as ubiquitination or acetylation as suggested by Bossis and Melchior (for review, see Ref. 28), resulting in distinct biochemical functions.

In summary, mounting evidence continues to imply that KAP1 may be an important component of DNA damage signaling pathway, and we demonstrate here that the Dox-induced KAP1 Ser-824 phosphorylation and KAP1 sumoylation switch could be a central regulatory circuit in mediating the de-repression of a subset of KAP1-repressed genes, such as p21, Gadd45α, Bax, Puma, and Noxa. With this caveat, the ATM-dependent inhibition of KAP1 sumoylation may represent a novel strategy of gene regulation with implications for other DNA damage agents, such as irradiation and cisplatin, by which ATM is also activated. The intricate interplay between ATM activation and the KAP1 sumoylation switch and their connection to stress signaling pathways are likely to be critical for the dynamic regulation of DNA damage responses.

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REFERENCES

1. Urrutia, R. (2003) Genome Biology 2004, 4, 231
2. Zheng, L., Pan, H., Li, S., Flesken-Nikitin, A., Chen, P. L., Boyer, T. G., and Lee, W. H. (2006) Mol. Cell 6, 757–768
3. Leong, Y. K., Thomas, S. N., Yang, A. J., and Ann, D. K. (2007) J. Biol. Chem. 282, 1595–1606
4. Schultz, D. C., Ayyananathan, K., Negorev, D., Maul, G. G., and Rauscher, F. J., III (2002) Genes Dev. 16, 919–922
5. Schultz, D. C., Friedman, J. R., and Rauscher, F. J., III (2001) Genes Dev. 15, 428–443
6. Underhill, C., Qutob, M. S., Yee, S. P., and Torchia, J. (2000) J. Biol. Chem. 275, 40463–40470
7. Lechner, M. S., Begg, G. E., Speicher, D. W., and Rauscher, F. J., III (2000) Mol. Cell. Biol. 20, 6449–6465
8. Srivathy, S. P., Stevens, J., and Schultz, D. C. (2006) Mol. Cell Biol. 26, 8623–8638
9. Okamoto, K., Kitabayashi, I., and Taya, Y. (2006) Biochem. Biophys. Res. Commun. 351, 216–222
10. Wang, C., Ivanov, A., Chen, L., Fredericks, W. J., Seto, E., Rauscher, F. J., III, and Chen, J. (2005) EMBO J. 24, 3279–3290
11. Shiloh, Y. (1997) Annu. Rev. Genet. 31, 635–662
12. Shiloh, Y. (2006) Trends Biochem. Sci. 31, 402–410
13. Sancar, A., Lindsey-Boltz, L. A., Unsai-Kacman, K., and Linn, S. (2004) Annu. Rev. Biochem. 73, 39–85
14. Manning, G., Whyte, D. B., Martinez, R., Hunter, T., and Sudarsanam, S. (2002) Science 298, 1912–1919
15. Kastan, M. B., and Bartek, J. (2004) Nature 432, 316–323
16. de-Deir, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) Cell 75, 817–825
17. Taylor, W. R., and Stark, G. R. (2001) Oncogene 20, 1803–1815
18. Vousden, K. H., and Lu, X. (2002) Nat. Rev. Cancer 2, 594–604
19. O’Geen, H., Suzzallo, S. L., Iyengar, S., Blahnik, K. J., Chang, H. Y., Green, R., and Farnham, P. J. (2007) PLoS Genet. 3, e89
20. White, D. G., Negorev, D., Peng, H., Ivanov, A. V., Maul, G. G., and Rauscher, F. J., III (2006) Cancer Res. 66, 11594–11599
21. Ziv, Y., Bielopolski, D., Galanty, Y., Lukas, C., Taya, Y., Schultz, D. C., Lukas, J., Bekker-Jensen, S., Bartek, J., and Shiloh, Y. (2006) Nat. Cell Biol. 8, 870–876
22. Ziv, Y., Bar-Shira, A., Pecker, I., Russell, P., Jorgensen, T. S., Tsarfati, I., and Shiloh, Y. (1997) Oncogene 15, 159–167
23. Nghiem, P., Park, P. K., Kim, Y., Yaziri, C., and Schreiber, S. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9092–9097
24. van de Wetering, M., Oving, I., Muncan, V., Pon Fong, M. T., Brantjes, H., van Leenen, D., Holstege, F. C., Brummelkamp, T. R., Agami, R., and Clevers, H. (2003) EMBO Rep. 4, 609–615
25. Oren, M. (2003) Cell Death Differ. 10, 431–442
26. Zhao, L. Y., Colosimmo, A. L., Liu, Y., Wan, Y., and Liao, D. (2003) J. Virol. 77, 11809–11821
27. Yun, J., and Lee, W. H. (2003) Mol. Cell. Biol. 23, 7305–7314
28. Bossis, G., and Melchior, F. (2006) Cell Div. 1, 13
29. Hay, R. T. (2005) Mol. Cell 18, 1–12
30. Johnson, E. S. (2004) Annu. Rev. Biochem. 73, 355–382
31. Hietakangas, V., Ahlkkos, J. K., Jakobsson, A. M., HELLEUSO, M., Sahib, N. M., holmberg, C. I., Mikhailov, A., Palvimo, J. J., Pirkkala, L., and Sistonen, L. (2003) Mol. Cell. Biol. 23, 2953–2968
32. Gregoire, S., Tremblay, A. M., Xiao, L., Yang, Q., Ma, K., Nie, J., Mao, Z., Wu, Z., Giguere, V., and Yang, X. J. (2006) J. Biol. Chem. 281, 4423–4433
33. Hietakangas, V., Anckar, J., Blomster, H. A., Fujimoto, M., Palvimo, J. J., Nakai, A., and Sistonen, L. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 45–50
34. Bossis, G., Malnou, C. E., Farras, R., Andermarcher, E., Hipskind, R., Rodriguez, M., Schmidt, D., Muller, S., Jariel-Encontre, L., and PIECHACZYK, M. (2005) Mol. Cell. Biol. 25, 6964–6979
35. Muller, S., Berger, M., Lehembre, F., Seeler, J. S., Haupt, Y., and Dejean, A. (2000) J. Biol. Chem. 275, 13321–13329
36. Lin, J. Y., Ohshima, T., and Shimotohno, K. (2004) FEBS Lett. 573, 15–18
37. Yang, S. H., Jaffray, E., Hay, R. T., and Sharrocks, A. D. (2003) Mol. Cell 12, 63–74
38. Nefkens, I., Negorev, D. G., Ishov, A. M., Michaelson, J. S., Yeh, E. T., Tanguay, R. M., Muller, W. E., and Maul, G. G. (2003) J. Cell Sci. 116, 513–524
39. Cheng, J., Perkins, N. D., and Yeh, E. T. (2005) J. Biol. Chem. 280, 14492–14498
40. Cheng, J., Wang, D., Wang, Z., and Yeh, E. T. (2004) Mol. Cell. Biol. 24, 6021–6028
41. Levine, A. J. (1997) Cell 88, 323–331
42. Vogelstein, B., Lane, D., and Levine, A. J. (2000) Nature 408, 307–310
43. Passalaris, T. M., Benanti, J. A., Gewin, L., Kiyono, T., and Galloway, D. A. (1999) Mol. Cell. Biol. 19, 5872–5881
44. Wang, Q., Fan, S., Eastman, A., Worland, P. J., Sausville, E. A., and O’Connor, P. M. (1996) J. Natl. Cancer Inst. 88, 956–965
45. Broude, E. V., Swift, M. E., Vivo, C., Chang, B. D., Davis, B. M., Kalurupalle, S., Blagosklonny, M. V., and Roninson, I. B. (2007) Oncogene 26, 6954–6958
46. Riballo, E., Kuhne, M., Rief, N., Doherty, A., Smith, G. C., Recio, M. J., Reis, C., Dahm, K., Fricke, A., Krempler, A., Parker, A. R., Jackson, S. P., Gennery, A., Jeggo, P. A., and Lobrich, M. (2004) Mol. Cell 16, 715–724