SERBP1 affects homologous recombination-mediated DNA repair by regulation of CtIP translation during S phase

Jang-Won Ahn1,†, Sunjik Kim1,†, Wooju Na1, Su-Jin Baek2,3, Jeong-Hwan Kim2, Keehong Min1, Jeonghun Yeom4,5, Hoyun Kwak6, Sunjoo Jeong6, Cheolju Lee4,5, Seon-Young Kim2,3 and Cheol Yong Choi1,*

1Department of Biological Sciences, Sungkyunkwan University, Suwon 440-746, Republic of Korea, 2Human Genomics Research Center, KRIBB, Daejeon 305-806, Republic of Korea, 3Department of Functional Genomics, University of Science of Technology, Daejeon 305-350, Republic of Korea, 4Center for Theragnosis, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea, 5Department of Biological Chemistry, University of Science of Technology, Daejeon 305-333, Republic of Korea and 6Department of Molecular Biology, Dankook University, Yongin 448-701, Republic of Korea

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ABSTRACT

DNA double-strand breaks (DSBs) are the most severe type of DNA damage and are primarily repaired by non-homologous end joining (NHEJ) and homologous recombination (HR) in the G1 and S/G2 phase, respectively. Although CtBP-interacting protein (CtIP) is crucial in DNA end resection during HR following DSBs, little is known about how CtIP levels increase in an S phase-specific manner. Here, we show that Serpine mRNA binding protein 1 (SERBP1) regulates CtIP expression at the translational level in S phase. In response to camptothecin-mediated DNA DSBs, CHK1 and RPA2 phosphorylation, which are hallmarks of HR activation, was abrogated in SERBP1-depleted cells. We identified CtIP mRNA as a binding target of SERBP1 using RNA immunoprecipitation-coupled RNA sequencing, and confirmed SERBP1 binding to CtIP mRNA in S phase. SERBP1 depletion resulted in reduction of polysome-associated CtIP mRNA and concomitant loss of CtIP expression in S phase. These effects were reversed by reconstituting cells with wild-type SERBP1, but not by SERBP1 ΔRGG, an RNA binding defective mutant, suggesting regulation of CtIP translation by SERBP1 association with CtIP mRNA. These results indicate that SERBP1 affects HR-mediated DNA repair in response to DNA DSBs by regulation of CtIP translation in S phase.

INTRODUCTION

DNA double strand breaks (DSBs) are the most serious lesion that can occur, since a single DSB can cause cell death if not repaired properly. Cells have developed elaborate mechanisms to cope with DSBs, in which DNA damage is recognized and repaired by the sequential action of damage sensors, signal transducers and effector molecules (1). DNA damage regulators are recruited to the site of DNA damage in an orchestrated fashion leading to a DNA damage response (DDR) such as cell cycle arrest, DNA repair, transcription activation and apoptosis, depending on cellular physiological status (2,3).

Different types of DNA repair pathways are activated depending on the cell cycle. In the G1 phase, DSBs are quickly repaired by non-homologous end joining (NHEJ). NHEJ is an error-prone solution, since the two DNA broken ends are ligated without dependence on a homologous template (4–6). In the presence of the sister chromatid in the S/G2 phase, DSBs are repaired by error-free homologous recombination (HR) in which the damaged DNA strand is replaced by using the sister chromatid as a template for second strand synthesis (7,8). Pathway choice is controlled by signaling cascades at the molecular level. In the G1 phase, RIF1 is recruited to the site of DNA damage for 53BP1 phosphorylation. This prevents DNA end resection and promotes NHEJ. In the S/G2 phase, CtIP becomes phosphorylated by CDK and associates with BRCA1. These proteins displace RIF1 and 53BP1, and recruit the MRN complex to initiate DNA end resection (9–14).

DNA end resection refers to the generation of 3’ overhang single strand DNA tails from the blunt ends of DNA.
SERBP1 over-expression was associated with high-grade tumor development (29) and high metastatic potential in lung cancer, indicating regulation of transcription, and DNA repair following camptothecin-induced DNA DSBs and is a critical step for HR-mediated DNA repair. CtIP is a central regulator of HR-mediated DNA repair in the S/G2 phase since endonuclease activity is required to produce short overhangs, followed by further resection by Exo1 and Dna2 nuclease to extend the short overhangs (15–19). Following DNA end resection, the single-strand-binding protein RPA2 is phosphorylated in an ATR- and CHK1-dependent manner. Phosphorylated RPA2 recognizes and binds to end-resected DNA, and is then replaced by Rad51 which forms nucleoprotein filaments and is involved in the search for homology and strand pairing with homologous DNA molecules (20, 21). Therefore, CtIP-derived DNA end resection is a critical step for HR-mediated DNA repair. Consistent with the cell cycle-specific role of CtIP in HR, CtIP expression is induced markedly during S phase. At the boundary of G1/S, CtIP expression is enhanced and regulated by the E2F/RB pathway (22) which is a main regulator for transcriptional activation of S phase-specific genes.

Serpine mRNA binding protein 1 (SERBP1) was identified as a binding protein to the 3' region of the mRNA encoding plasminogen activator inhibitor (PAI) type I, suggesting regulation of PAI mRNA stability (23). Although there is little evidence for a functional relationship, amino acid sequence homology suggests that SERBP1 is a member of the HABP4 family which has the hyaluronan binding domain and an RNA binding motif in common. SERBP1 does not contain RNA recognition motif (RRM) or K homology (KH) domain, but has an arginine-glycine (RG)-rich and arginine-glycine-glycine (RGG) box for target mRNA binding (24). SERBP1 interacting proteins were identified using the yeast two-hybrid system. Interestingly, although SERBP1 predominantly localizes to the cytoplasm, a number of nuclear proteins, such as CHD3, Daxx, Topors and PIA/Sy, were identified as SERBP1-interacting proteins (25, 26). SERBP1 has been implicated in tumorigenesis and resistance to anti-cancer drugs. Over-expression of SERBP1 has been observed in various cancers including acute lymphoblastic leukemia (27), breast cancer (28), ovarian carcinoma (29, 30), glioblastoma (31) and squamous lung-cell carcinoma (32). In the case of ovarian and non-small cell lung cancer, SERBP1 over-expression was associated with high grade tumor development (29) and high metastatic potential (33), respectively. Furthermore, in various human cancer cell lines, cells that overcome a cisplatin challenge to become cisplatin resistant also expressed increased levels of SERBP1 (34, 35). Although a number of studies suggest that SERBP1 may be involved in regulation of transcription, RNA metabolism, cell proliferation and apoptosis, specific cellular functions of SERBP1 remain to be uncovered. In particular, although SERBP1 is an RNA binding protein containing RNA binding motifs and interacts with other RNA binding proteins in an RNA binding-dependent manner in stress granules (36), little is known about the functional target RNAs of SERBP1.

Since CtIP plays a critical role in HR-mediated DNA repair, the increase of CtIP expression in S phase is important in the choice of repair pathway and maintenance of chromosome integrity during S phase. Although CtIP transcription was reported to be enhanced by the E2F/RB pathway at the boundary of G1/S phase, transcriptional regulation is not sufficient to explain the marked induction of CtIP protein levels compared to the modest increase in CtIP mRNA levels (22). Here, we show that SERBP1 binds to CtIP mRNA and enhances CtIP expression at the translational level in S phase. In SERBP1-depleted cells, levels of polysome-associated CtIP mRNA were reduced and thereby CtIP expression was abrogated in S phase. Therefore, SERBP1 affects HR-mediated DNA end resection, checkpoint activation, and DNA repair following camptothecin-induced DNA DSBs by regulation of CtIP expression at the translational level.

MATERIALS AND METHODS

Plasmid construction

SERBP1 was constructed by RT-PCR from mRNAs prepared from HCT116 cell extract. A DNA fragment encoding SERBP1 was cloned into the pCEFL-GST mammalian expression vector. EGFP-SERBP1 WT was constructed by insertion of DNA fragment encoding SERBP1 into the EcoRI/BamHI sites of pEGFP-C2. EGFP-SERBP1 ΔR (deletion of 126–137), ARG (deletion of 163–184) and ΔRG (deletion of 343–359) mutants were generated using wild-type EGFP-SERBP1. All constructs were verified by DNA sequencing. A cDNA fragment containing the 5’-3’-UTR and coding region of CtIP was prepared by RT-PCR from mRNA and inserted into the NheI and BamHI sites of the pcI mammalian expression plasmid to generate 5’/3’UTR-CtIP. The expression plasmid 3’UTR-CtIP was constructed with the same strategy with the SV40 poly(A) sequences replaced by the CtIP 3’-UTR. To construct the plasmid containing 5’UTR-CtIP and CtIP with no UTRs, each corresponding cDNA fragment was inserted into the NheI and XbaI sites of the pcI plasmid. In these plasmids, the SV40 poly(A) sequences are utilized for CtIP expression.

Antibodies and other materials

Rabbit anti-phospho-Chk1 (pS345, #2341), anti-phospho-Chk2 (pT68, #2661), anti-H2AX (#2595), anti-Chk1 (#2360), anti-Chk2 (#3440), anti-UBC13 (#4919S) and mouse anti-Cyclin E (#4129) were purchased from Cell Signaling Technology. Rabbit anti-CtIP (A300–488A) and anti-phospho-RPA2 (p4/8, A300-245A) were purchased from Abcam. Mouse anti-Actin (ab8227), anti-RAD52 (ab18264) and anti-RAD54B (ab137584) were purchased from Abcam, and anti-green fluorescent protein (GFP; 632381) was purchased from Clontech Laboratories. Anti-RAD51 (sc-8349) and anti-XRCC2 (sc-5895) were purchased from Santa Cruz Biotechnology. Rabbit anti-SERBP1 antibody was raised against a peptide containing the SERBP1 sequence (186-SRGKREFDRHSGSDRS-202).

Cell culture, and cell lines expressing GST-SERBP1 or GFP-SERBP1

U2OS and HeLa cells were grown in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal bovine
serum. U2OS cells were pre-synchronized at G1 phase by serum-starvation for 48 h. This was followed by incubation with 0.4 µg/ml aphidicolin for 20 h. Alternatively, U2OS cells were synchronized at early S phase by incubation with 1.6 µg/ml aphidicolin for 20 h. Double strand breaks in S phase were induced by treatment with 1 µM of CPT for 2 h. To generate cell lines stably expressing GST-SERBP1, EGFP-SERBP1 WT and deletion mutants, expression plasmids encoding GST-SERBP1, siRNA-resistant EGFP-SERBP1 WT and deletion mutants were transfected into HeLa cells and U2OS cells, respectively, and transfected cells were selected on G418-containing media for 2 weeks. The selected clones were maintained on 600 µg/ml of Genetecin (Gibco). Stable expression was regularly monitored by immunoblotting with anti-SERBP1 and anti-GFP antibodies.

RT-qPCR
TRizol (Invitrogen) was used to extract RNA. Reverse transcription was performed according to the manufacturer’s protocol (iQ SYBR® Green Supermix, Bio-Rad Laboratories). PCR primers for detecting CtIP mRNA were 5′-AGATCGGTTAAGAGCAGGCTT-3′ (sense) and 5′-GATTCGTGCAGGATATT-3′ (antisense), and actin mRNA were 5′-ACC-GAGCGGCTACAG-3′ (sense) and 5′-CTTAAATGTACCCAGATTTC-3′ (antisense). Real-time quantitative PCR (qPCR) analysis was performed using CFX96 Real-Time PCR Systems (Bio-Rad Laboratories).

Immunoblotting
Immunoblotting was performed as previously described (37). Total cell extracts were prepared by scraping cells in Laemmli buffer (0.8% SDS, 4% glycerol, 280 mM β-mercaptoethanol, 25 mM Tris–HCl, pH 6.8). Proteins were resolved by SDS-PAGE, transferred onto PVDF membranes (Millipore) and probed using the appropriate primary and secondary antibodies coupled to horseradish peroxidase (HRP; Dako-Pierce). Detection was performed using CPT 1 M for 1–2 h before being fixed with 4% paraformaldehyde for 5 min and incubated with the appropriate antibody. Fluorescence microscopy was conducted with a Zeiss Axioskop 2 microscope; excitation wavelengths of 543 nm (rhodamine red) and 488 nm (GFP) were used.

The acquired images were processed with Adobe Photoshop. For RPA immunofluorescence, permeabilization was performed prior to fixation in pre-extraction buffer (10 mM PIPES pH 7.0, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl₂, 0.5% Triton-X100) for 10 min at room temperature. Samples were then blocked in 5% bovine serum albumin and immunostained with the appropriate primary and secondary antibodies. Secondary antibodies coupled to AlexaFluor 488 and 594 were purchased from Molecular Probes.

Homologous recombination (HR) assays
A HeLa cell line containing an HR reporter (HeLa-DR-GFP) was used. One day after siRNA transfection, HeLa-DR-GFP cells were co-transfected with an I-SceI expressing vector (pCBA-I-SceI). After 48 h, cells were collected and subjected to FACS analysis to determine HR efficiency. Cells that were dually positive for GFP were measured for all siRNA or treatments, background was subtracted and the final results were normalized to control siRNA.

Immunoprecipitation of RNP complex (RIP) and RNA sequencing
For immunoprecipitation (IP) of endogenous RNP complexes from whole-cell extracts, cells were lysed in 20 mM Tris–HCl at pH 7.5, 100 mM KCl, 5 mM MgCl₂, and 0.5% NP-40 for 10 min on ice and centrifuged at 10 000 × g for 15 min at 4°C. The supernatants were incubated with protein A-Sepharose beads coated with anti-SERBP1 antibody or anti-GFP antibody or control IgG (Santa Cruz Biotechnology) for 1 h at 4°C. After the beads were washed with NT2 buffer (50 mM Tris–HCl at pH 7.5, 150 mM NaCl, 1 mM MgCl₂, and 0.05% NP-40), the complexes were incubated with 20 U of RNase-free DNase I (15 min at 37°C) and further incubated with 0.1% SDS/0.5 mg/ml Proteinase K (15 min at 55°C) to remove DNA and proteins, respectively. The RNP isolated from IP was further assessed by RT-qPCR analysis. RIP for RNA sequencing of endogenous targets of GST-SERBP1 in the S phase was performed using the HeLa-GST-SERBP1 stable cell line and GST-coated beads. Asynchronous HeLa-GST-SERBP1 cells and double thymidine-blocked, S phase-arrested HeLa-GST-SERBP1 cells were lysed, and pull-down was performed using GST-coated beads. RNA from the two RIP samples (asynchronous and S phase) were converted to cDNA libraries according to the Illumina TruSeq RNA Sample Prep Guide. The HeLa-GST-SERBP1 cell RIP-seq libraries were sequenced in 2 × 100 bp using the Illumina HiSeq-2000 platform. Then, S phase-specific SERBP1 target mRNAs were identified by comparing the asynchronous sample with the S phase-arrested sample (Supplementary Table S1). Target mRNAs enriched in S phase were classified by using the DAVID ontology system to obtain HR related genes (Supplementary Table S2).

Polysome profile analysis
U2OS cells (5 × 10⁶ per sample) at ~80% confluence were incubated for 15 min in 0.1 mg/ml cycloheximide, then lifted by scraping in 1 ml PEB lysis buffer (0.3 M NaCl, 15
mM MgCl₂, 15 mM Tris- HCl, pH 7.6, 1% Triton X-100, 1 mg/ml heparin, and 0.1 mg/ml cycloheximide) and lysed on ice for 10 min. Nuclei were pelleted (10 000 × g, 10 min) and the resulting supernatant was fractionated through a 10–50% linear sucrose gradient. After centrifugation (Beckman SW55, 38 000 rpm, 120 min, 4°C), the material was fractionated into 0.5 ml fractions. TRIzol LS (Invitrogen) was used to isolate RNA from each fraction, and half of the RNA isolated from each fraction was used for RT-qPCR.

In vivo labeling of Ctp

U2OS cells were mock-depleted or SERBP1-depleted and transfected with the CtIP expression plasmid containing the 5'- and 3'-UTR along with GFP expression plasmid as a negative control. Transfected cells were synchronized at S phase by treatment with aphidicolin, followed by a 24 h incubation in culture media containing 35S-methionine and 35S-cysteine. Cell lysates were immunoprecipitated with anti-CtIP and anti-GFP antibodies, and the proteins were resolved in 10% SDS-PAGE. Proteins were transferred to PVDF membrane and the blots were exposed in a Phosphoimager and utilized in immunoblotting using anti-CtIP and anti-GFP antibodies.

RESULTS

SERBP1 is crucial in ATR-dependent double strand break signaling

SERBP1 is over-expressed in various cancers including ovarian cancer, breast cancer, non-small cell lung carcinoma, glioblastoma and acute lymphoblastic leukemia (27–31,33). SERBP1 is associated with the stress response, since it is a component of stress granules induced by hydrogen peroxide (36). In addition, SERBP1 is over-expressed in cells exhibiting resistance to the anti-cancer drug cisplatin (35). Therefore, we were curious whether SERBP1 is involved in the DNA damage response induced by DNA DSBs.

To investigate the functional relevance of SERBP1 in the DDR, the viability of SERBP1-depleted cells was examined upon exposure to DSBR-inducing agents. SERBP1-depleted HeLa cells were exposed to increasing doses of ionizing radiation (IR) or the topoisomerase I inhibitor camptothecin (CPT), and cell survival rates were measured by counting the number of colonies. We found that SERBP1 depletion rendered cells more sensitive to IR and CPT (Figure 1A and B), suggesting that SERBP1 is involved in cellular responses to DSBs. Administration of cells with CPT induces DSBs only during S phase of the cell cycle, whereas ionizing radiation induces DSBs throughout the cell cycle. In this study, we addressed the role of SERBP1 in the cellular response to CPT-mediated DNA DSB in S phase.

To determine the effects of SERBP1 depletion on the DDR to CPT treatment at the molecular level, checkpoint signaling including phosphorylation of CHK1, CHK2 and H2AX was examined by immunoblotting in SERBP1-depleted cells. Interestingly, CHK1 phosphorylation was significantly impaired in SERBP1-depleted cells following CPT treatment (Figure 1C and D), whereas phosphorylation of CHK2 and H2AX were not affected (Figure 1C and D), demonstrating that SERBP1 is crucial for ATR-dependent DSB signaling, and not for ATM-dependent phosphorylation of H2AX and CHK2. Consistent with DSBs induced during S phase being the major cytotoxic lesions caused by camptothecin, SERBP1 depletion did not affect DNA damage-induced CHK1 phosphorylation in cells arrested at early S phase by treatment with high doses of the DNA-replication inhibitor aphidicolin (Figure 1D, lanes 2 and 5). These data suggested that SERBP1 depletion impairs cell survival when DSBs are generated during S phase.

SERBP1 is involved in homologous recombination-mediated DNA repair during S phase

DSBs are generated when replication forks encounter DNA lesions, and are repaired by homologous recombination in S phase. Defects in CHK1 activation by SERBP1 depletion led us to examine if DNA end resection of DSBs occurs by phosphorylation of RPA2, an upstream marker for ATR-dependent CHK1 activation. Immunoblotting with anti-phospho-RPA2 antibody following CPT treatment indicated that DNA damage-induced phosphorylation of the RPA2 subunit on Ser4 and Ser8 was markedly reduced upon SERBP1 depletion (Figure 2A). Consistently, formation of DNA damage-induced RPA2 foci was defective in cells where γ-H2AX foci were normally detected at the sites of DNA damage (Figure 2B). These data indicate that SERBP1 depletion impairs RPA2 phosphorylation and foci formation following CPT treatment. Next, the cellular response to CPT-induced DNA damage was determined using the DR-GFP system, which consists of direct repeats of mutated GFP (38), upon SERBP1 depletion. Consistent with the defects in CHK1 and RPA2 activation upon SERBP1 depletion, a chromosomal I-SceI-mediated gene conversion assay indicated that the efficiency of HR was markedly reduced to 42% of the control, comparable to the effect of depleting CtIP as a positive control (19) (Figure 2C). From these data, we conclude that SERBP1 is involved in RPA activation and HR-mediated DNA repair during S phase.

SERBP1 RGG box is required for DNA end resection of DSBs

Next, we determined which functional domain(s) of SERBP1 regulates DNA end resection and HR. An expression plasmid encoding GFP-SERBP1 was mutated to be resistant to knockdown by SERBP1 siRNA for reconstruction experiments. This plasmid was further mutated to delete the R-rich, RG-rich or RGG-box (Figure 3A, left panel). To address the functions of the SERBP1 deletion mutants, cell lines expressing each SERBP1 deletion mutant were established. Expression of SERBP1 siRNA in these cell lines caused depletion of endogenous SERBP1, but not the chromosome-integrated GFP-SERBP1 deletion mutants (Figure 3A, right panel). Localization of the SERBP1 deletion mutants was not altered by deletion of each SERBP1 domain (Supplementary Figure S1). Expression levels of chromosome-integrated siRNA-resistant GFP-SERBP1 and deletion mutant proteins were similar to...
Figure 1. SERBP1 is required for the ATR-dependent signaling pathway in response to CPT treatment. (A and B) SERBP1 depletion causes hypersensitivity to ionizing radiation (IR) and camptothecin (CPT). SERBP1-depleted and mock-depleted HeLa cells were exposed to increasing doses of IR (A) or CPT (B). Cell survival rates were determined by counting colony numbers. Data are presented as the mean ± SEM. (C) SERBP1 depletion results in impairment of CHK1 phosphorylation upon CPT treatment. SERBP1-depleted and mock-depleted U2OS cells were left untreated or treated with CPT, followed by immunoblotting using the indicated antibodies. (D) SERBP1 depletion does not affect aphidicolin-induced DNA damage and CHK1 phosphorylation. SERBP1-depleted and mock-depleted U2OS cells were left untreated or treated either with CPT or aphidicolin, followed by immunoblotting using the indicated antibodies.

The levels of endogenous SERBP1, suggesting that the reconstitution approach we took is physiologically relevant. Immunostaining of cells indicated that RPA2 phosphorylation and RPA2 foci formation was impaired by SERBP1 depletion following CPT treatment. Reconstitution of cells with wild-type SERBP1 restored RPA2 phosphorylation and RPA2 foci formation. However, cells reconstituted with GFP-SERBP1 ΔRGG, but not GFP-SERBP1 ΔR or ΔRG, were defective in CPT-induced RPA phosphorylation and formation of RPA foci (Figure 3B and C). Similar results were observed regarding CHK1 phosphorylation (Supplementary Figure S2). We were able to rule out off-target effects of the siRNA against SERBP1, since defects in RPA2 phosphorylation by depletion of endogenous SERBP1 was recovered by expression of siRNA-resistant wild-type SERBP1, but not by expression of the siRNA-resistant SERBP1 ΔRGG mutant, which is an RNA binding defective mutant. To address the functional contribution of SERBP1 RGG-box to cell survival upon CPT treatment, endogenous SERBP1 was depleted in SERBP1 mutant-expressing cells, followed by CPT administration. SERBP1 depletion resulted in reduction of cell survival rate. However, cells reconstituted with SERBP1 wild-type, ΔR, or the ΔRG mutant showed a recovery in survival rate, but not cells reconstituted with the SERBP1 ΔRGG mutant (Figure 3D). Since the RGG box is an RNA binding motif, the RNA-binding activity of SERBP1 is required for RPA2-
SERBP1 is involved in RPA activation and homologous recombination in response to CPT treatment. (A) SERBP1 depletion results in impairment of RPA2 phosphorylation. SERBP1-depleted and mock-depleted U2OS cells were left untreated or treated with CPT, followed by immunoblotting using the indicated antibodies. (B) SERBP1 depletion abrogates RPA2 foci formation, not γ-H2AX foci formation. SERBP1-depleted and mock-depleted U2OS cells were left untreated or treated with CPT, followed by immunostaining using the indicated antibodies. The percentage of γ-H2AX foci positive cells with RPA2 foci is depicted on the right. Data are presented as the mean ± SEM. (C) Defects in HR-mediated DNA repair by SERBP1 depletion. HeLa cells containing direct repeat of mutated GFP (DR-GFP) were mock-depleted or depleted of SERBP1, following induction of DSBs by transfection of the I-SceI expression plasmid. The intensity of GFP fluorescence, an indicator of HR, was analyzed by FACS. HR efficiencies are depicted on the right. Data are presented as the mean ± SEM. Immunoblot indicates SERBP1 depletion in DR-GFP HeLa cells. All experiments were conducted in parallel with CtIP depletion as a positive control of HR.

mediated DNA repair and regulation of cell survival upon CPT treatment.

SERBP1 binds to CtIP mRNA and induces CtIP expression during S phase

Since SERBP1 regulates DNA end resection through the RGG box which contains RNA binding activity, we hypothesized that SERBP1 affects DNA end resection by regulation of factor(s) which participate in HR. To identify the target mRNAs of SERBP1, RNA immunoprecipitation-coupled RNA sequencing (RIP-Seq) was performed using cytoplasmic lysates from asynchronous or S phase HeLa-GST-SERBP1 stable cell lines with glutathione beads. Using an RIP-Seq approach, we identified 6900 target mRNAs of SERBP1 which show enhanced binding at S phase (Supplementary Table S1). Gene ontology analyses of S phase-enriched SERBP1 target genes indicate that nine genes relevant to HR were identified (Figure 4A and Supplementary Table S2). We confirmed that SERBP1 binds to the mRNAs of the HR-related genes by qPCR following immunoprecipitation of GFP-SERBP1 WT and ΔRGG mutant (Supplementary Figure S3). Among the nine HR-related genes, we focused on CtIP (CtBP-interacting protein) for further
study, since CtIP is a critical player in the regulation of HR and the most upstream regulator of HR among the nine targets identified. We performed experiments to confirm whether CtIP mRNA is a binding target of SERBP1. As shown in Figure 4B, CtIP mRNA was enriched more than eightfold in SERBP1 immunoprecipitation (IP) samples compared with control IgG IP samples. This enrichment was enhanced in cells arrested at S phase by an aphidicolin block (Figure 4C). In addition, binding to CtIP mRNA was not observed with SERBP1 ΔRGG, indicating that SERBP1 binding to CtIP is mediated by the RGG-box. These data indicate that SERBP1 associates with CtIP mRNA in S phase.

Since the effects of SERBP1 depletion on RPA phosphorylation are similar to the effects of CtIP depletion, we examined the effects of SERBP1 depletion on CtIP protein levels. To determine the effects of SERBP1 depletion, SERBP1 depleted cells were arrested at G1 and S phase by
Figure 4. Identification of CtIP mRNA as an SERBP1 binding target, and SERBP1 binding to CtIP mRNA in an S phase-specific manner. (A) SERBP1 RNA binding targets relevant to HR. List of SERBP1 binding targets relevant to HR, identified by RIP-seq analysis. (B) SERBP1 binding to CtIP mRNA. U2OS cell lysates were immunoprecipitated with control IgG or anti-SERBP1 antibody, followed by quantitation of associated CtIP mRNA using qPCR. Data are presented as the mean ± SEM. (C) SERBP1 binds to CtIP mRNA in S phase through the RGG box. Parental U2OS cells and U2OS cells expressing wild-type GFP-SERBP1 or the GFP-SERBP1 ΔRGG mutant were left untreated or treated with aphidicolin to arrest the cell cycle in S phase. U2OS cells expressing wild-type GFP-SERBP1 or the GFP-SERBP1 deletion mutants were depleted of endogenous SERBP1 before immunoprecipitation with anti-GFP antibody. Cell lysates were prepared from these cells and immunoprecipitated with anti-GFP antibody, followed by quantitation of associated CtIP mRNA using qPCR. Fold difference of SERBP1-associated CtIP mRNA abundance after normalization of input CtIP mRNA is depicted on the graph. Data are presented as the mean ± SEM.

exposure to serum starvation and aphidicolin treatment, respectively, and cells were harvested at the indicated time points after release from cell cycle arrest. Immunoblotting indicated that CtIP protein levels were not induced during S phase in SERBP1 depleted cells, whereas CtIP was induced during S phase in control cells (Figure 5A and B). The reduction of CtIP expression was not due to proteasomal degradation, since CtIP levels were not recovered by MG132 treatment (Figure 5C). Also, siRNA-mediated depletion of SERBP1 did not alter cell-cycle distribution profiles (Supplementary Figure S4). Next, we examined whether CtIP expression is induced by SERBP1 at the transcriptional level and/or by regulation of mRNA stability. Quantitative real-time PCR (qPCR) indicated that CtIP mRNA levels are not altered upon SERBP1 depletion, suggesting that the reduced CtIP expression is not due to changes in CtIP mRNA levels (Figure 5D). To determine whether the reduction in CtIP levels upon SERBP1 depletion is due to changes in mRNA stability, the CtIP mRNA half-life (t1/2) was analyzed following treatment of cells with actinomycin D to inhibit de novo mRNA synthesis. CtIP mRNA levels, normalized to GAPDH mRNA, were determined by RT-qPCR. CtIP mRNA stability was not affected by SERBP1 depletion (Figure 5E). These data indicate that SERBP1 does not affect the synthesis and stability of CtIP mRNA.
Figure 5. SERBP1 depletion results in loss of CtIP expression during S phase. (A and B) SERBP1 depletion results in loss of CtIP expression during S phase. U2OS cells were left undepleted or depleted of SERBP1 and subjected to serum starvation (SS) for 36 h (A) or treated with aphidicolin (Aph) to arrest the cell cycle in S phase (B). U2OS cell were released from cell cycle arrest and harvested at the indicated time points. Cell lysates were analyzed by immunoblotting using the indicated antibodies. (C) Loss of CtIP expression by SERBP1 depletion is not due to proteasomal degradation. U2OS cells were left undepleted, depleted of SERBP1, or treated with MG132 for 1 h following SERBP1 depletion. Cell lysates were analyzed by immunoblotting using the indicated antibodies. (D) CtIP mRNA levels are not altered by SERBP1 depletion. U2OS cells were left undepleted or depleted of SERBP1. Cell lysates were prepared and mRNA was isolated, followed by RT-PCR. The levels of mRNA were determined by electrophoresis on a 1% agarose gel. The band intensities are depicted on the right. Data are presented as the mean ± SEM. A and S indicates asynchronous and S phase, respectively. (E) CtIP mRNA stability is not affected by SERBP1 depletion. U2OS cells were left undepleted or depleted of SERBP1 and cells were treated with aphidicolin to arrest the cell cycle in S phase. The cell were released from S phase by removing aphidicolin in the presence of actinomycin D to inhibit de novo synthesis of CtIP mRNA. Cells were harvested at the indicated time points and the levels of CtIP mRNA were determined by qPCR following mRNA preparation from cell lysates.

SERBP1 is required for CtIP translation during S phase

Next, we performed polysome profiling analysis to determine whether CtIP translation is affected by SERBP1. Cytoplasmic extracts from G1 and S phase cells were fractionated through sucrose density gradient centrifugation, with the lightest components sedimenting at the top (fractions 1 and 2), the small (40S) and large (60S) ribosomal subunits and monosomes (80S) in fractions 3–5, and progressively larger polysomes in fractions 6–10 as shown by rRNA patterns (Figure 6A). Quantitation of CtIP mRNA in each fraction indicated that actively translated CtIP mRNA associated with polysome was decreased by SERBP1 depletion in S phase (Figure 6B), while translation of CtIP mRNA in G1 phase was not affected (Figure 6A). These results indicate that basal level CtIP translation in G1 phase is not affected by SERBP1 depletion, but translational induction of CtIP in S phase is mediated by SERBP1. Consistently, SERBP1 depletion at S phase caused a decrease in CtIP levels which is dependent on the SERBP1 RGG domain (Supplementary Figure S5), while SERBP1 depletion in G1 phase did not affect basal CtIP levels (Figure 6D). To further confirm that SERBP1-mediated regulation of CtIP translation is associated with SERBP1 binding to CtIP mRNA, CtIP translation was determined with cell lysates prepared from cells expressing the SERBP1 ΔRGG mutant following endogenous SERBP1 depletion. Consistent with the results from SERBP1 depletion, CtIP translation in S phase was not enhanced in cells expressing the SERBP1 ΔRGG mutant (Figure 6C). Also consistently, the SERBP1 ΔRGG mutant did not allow recovery of CtIP levels following depletion of endogenous SERBP1 in S phase, in contrast to wild-type SERBP1 (Figure 6E). Taken together, these findings indicate that SERBP1 binding to CtIP mRNA through the RGG box is required for translational induction of CtIP in S phase.

The CtIP 3′-UTR is required for SERBP1 regulation of CtIP expression at the translational level during S phase

To determine which region of CtIP mRNA is required for SERBP1-mediated translational control, siRNA-resistant CtIP expression plasmids containing the 5′-UTR and/or 3′-UTR were constructed (Figure 7A). siRNA-resistant CtIP expression plasmids were transfected into U2OS cells with endogenous CtIP depleted using siRNA against CtIP, so that expressed CtIP originates from the transfected CtIP

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Figure 6. SERBP1 induces CtIP expression at the translational level in S phase. Graph and inset indicate % mRNA and relative mRNA, respectively. (A and B) CtIP translation is induced by SERBP1 in the S phase, but not in the G1 phase. U2OS cells were synchronized at the G1 (A) and S phase (B) by serum starvation and aphidicolin treatment, respectively. The synchronized cells were left undepleted or depleted of SERBP1. Cell lysates were prepared and subjected to polysome profiling. rRNA in each fraction was separated on a 1% agarose gel as depicted on the left. (C) Translational induction of CtIP mRNA is mediated by the SERBP1 RGG-box. U2OS cells expressing siRNA-resistant wild-type SERBP1 or SERBP1 ΔRGG were depleted of endogenous SERBP1 and treated with aphidicolin to arrest the cell cycle in S phase. Cell lysates were prepared and subjected to polysome profiling. rRNA in each fraction was separated on a 1% agarose gel as depicted on the left. (D) U2OS cells were synchronized at the G1 and S phase by serum starvation and aphidicolin treatment, respectively. The synchronized cells were left undepleted or depleted of SERBP1. Cell lysates were subjected to immunoblotting using the indicated antibodies. The band intensities of CtIP to Actin are depicted on the right. (E) U2OS cells expressing siRNA-resistant wild-type SERBP1 or SERBP1 ΔRGG were depleted of endogenous SERBP1 and treated with aphidicolin to arrest the cell cycle in S phase. Cell lysates were subjected to immunoblotting using the indicated antibodies. The band intensities of CtIP to Actin are depicted on the right. The asterisk indicates a nonspecific band.
Figure 7. The CtIP 3′-UTR is required for SERBP1 binding and regulation of CtIP expression by SERBP1 at the translational level during S phase. (A) A schematic representation of the siRNA resistant CtIP expression plasmids, and summary of SERBP1 binding and regulation of CtIP by SERBP1. Asterisks indicate point mutations which render expressed mRNA resistant to siRNA against CtIP. (B) CtIP depleted U2OS cells were transfected with each siRNA-resistant CtIP and GFP expression plasmids, and cells were arrested at G1 or S phase by incubation of cells with serum-free media for 48 h or aphidicolin-containing media for 24 h, respectively. Cell lysates were analyzed by immunoblotting with the indicated antibodies. (C and D) CtIP-depleted U2OS cells were transfected with each siRNA-resistant CtIP and GFP expression plasmids, and cells were arrested at G1 phase (C) or S phase (D) in the presence of control siRNA or siSERBP1. Cell lysates were analyzed by immunoblotting with the indicated antibodies. (E) A CtIP expression plasmid containing both the 5′- and 3′-UTR was transfected into U2OS cells along with the GFP expression plasmid, and cells were arrested at S phase in the presence of control siRNA or siSERBP1. S phase cells were incubated with culture media containing 35S-methionine and 35S-cysteine for 24 h. Cell lysates were immunoprecipitated with anti-CtIP and anti-GFP, and the precipitated proteins were resolved in 10% SDS-PAGE followed by transfer to PVDF membrane. The membrane blot was utilized to quantitate 35S-labeled CtIP and GFP using a phosphor-imager, and utilized in immunoblotting using anti-CtIP and anti-GFP antibodies. (F) CtIP-depleted U2OS cells expressing GFP-SERBP1 were transfected with CtIP expression plasmids containing the 5′-UTR and/or 3′-UTR followed by administration of aphidicolin for 24 h to arrest the cell cycle at S phase. Cell lysates were immunoprecipitated with anti-GFP antibody followed by quantitation of associated CtIP mRNA using qPCR. Fold difference of SERBP1-associated CtIP mRNA abundance after normalization of input CtIP mRNA is depicted on the graph. Data are presented as the mean ± SEM.

expression plasmids. Comparison of CtIP expression indicated that CtIP expression was induced at S phase when CtIP expression plasmids containing the 3′-UTR (both 5′/3′-UTR-CtIP and 3′-UTR-CtIP) were transfected, but not when plasmids without the 3′-UTR were transfected (Figure 7B). Furthermore, in SERBP1 depleted cells, CtIP expression was inhibited in the S phase, but not in the G1 phase when CtIP expression plasmids containing the 3′-UTR (both 5′/3′-UTR-CtIP and 3′-UTR-CtIP) were transfected. This effect was not observed when CtIP expression plasmids without the 3′-UTR were transfected into U2OS cells (Figure 7C and D). Since CtIP expression from the plasmid containing both the 5′- and 3′-UTR mimics endogenous CtIP expression, we confirmed SERBP1-mediated translational control of CtIP expression using 35S-methionine/cysteine incorporation. U2OS cells transfected with the 5′- and 3′-UTR CtIP expression plasmid were synchronized at S phase and the levels of 35S-methionine/cysteine incorporated into de novo synthesized CtIP were analyzed. Consistent with SERBP1-mediated translational control, 35S-CtIP levels were markedly reduced by SERBP1 depletion, but 35S-GFP levels were not (Figure 7E), confirming translational regulation of CtIP by SERBP1. Next, we addressed whether SERBP1 binds to
the 3′-UTR of CtIP mRNA. To test this, a set of CtIP expression plasmids containing the 5′-UTR and/or 3′-UTR were transfected into U2OS cells expressing GFP-SERBP1, and CtIP mRNAs bound to GFP-SERBP1 were analyzed. Analysis using real-time qPCR indicated that CtIP mRNA containing the 3′-UTR bound strongly to GFP-SERBP1 (Figure 7F). These results indicate that SERBP1 binds to CtIP 3′-UTR to regulate expression of CtIP at the translational level during S phase.

**DISCUSSION**

Cells are continuously exposed to physical and/or chemical factors inducing DNA damage, thereby cellular DNA undergoes various types of mutations. Cells have developed elaborate repair mechanisms depending on the type of mutations. DSBs, the most severe type of mutation, are repaired by NHEJ or HR depending on the cell cycle. Consistent with the role of CtIP in HR, CtIP expression is enhanced in S phase. Induction of CtIP expression occurs at the transcriptional level at the G1/S transition boundary. E2F binding sites were found in the CtIP promoter and phosphorylation of RB by CDK at the boundary of G1/S transition results in increase of CtIP mRNA levels in S phase (22). However, there is discrepancy between CtIP mRNA and protein levels in G1 and S phase, in which CtIP mRNA increases slightly at the G1/S boundary while CtIP protein levels markedly increase during S phase. These results suggest that other mechanisms may be involved in regulating CtIP expression at the G1/S transition in addition to transcriptional induction. In this study, we demonstrate that CtIP expression is regulated at the translational level by recruitment of SERBP1 to CtIP mRNA. First, polysome profiling using sucrose density gradient centrifugation indicated that polysome-associated CtIP mRNA was increased in S phase (Figure 6A), suggesting the translational induction of CtIP expression in S phase. Second, depletion of SERBP1 markedly reduced polysome-associated CtIP mRNA and resulted in reduction of CtIP protein levels (Figure 6B and D). Third, reconstitution of siRNA-resistant wild-type SERBP1 restored CtIP translation following endogenous SERBP1 depletion, while SERBP1 ΔRGG, an RNA binding defective mutant, did not (Figure 6C and E). Depletion of SERBP1 did not affect the basal expression of CtIP in G1 phase, but induction of CtIP expression in S phase was abrogated by SERBP1 depletion. Therefore, CtIP expression is regulated at both the transcriptional and translational levels. This mechanism ensures efficient upregulation of CtIP expression during S phase. Regulation of CtIP expression at the translational level may be advantageous to quickly adjust the protein levels of CtIP depending on physiological requirements during S phase.

Following the initial discovery of SERBP1 as a PAI-I mRNA binding protein, binding partners of SERBP1 were identified by yeast two-hybrid screening and coimmunoprecipitation approaches (25,26,39). However, little is known of the targets of SERBP1 as an RNA binding protein and the physiological role of SERBP1 under normal and DNA damage conditions. CtIP is the first target of SERBP1-mediated translational control. In the absence of SERBP1, CtIP mRNA level was not altered, but CtIP protein level was decreased, since CtIP translation induction was disrupted during S phase. Consistent with this result, SERBP1 depletion resulted in the same defects caused by CtIP depletion, such as impairment of RPA2 phosphorylation and CHK1 activation during the cellular response to camptothecin-induced DSBs. Determination of homologous recombination rate using the DR-GFP system indicate that SERBP1 depletion reduced the rate of HR, comparable to the rate upon CtIP depletion. To our knowledge, this is the first report that a component of HR-mediated DNA repair is regulated at the translational level. During the course of this study, we identified other HR-associated genes as a potential SERBP1 targets (Figure 4A). We focused on the functional analysis of SERBP1 association with CtIP mRNA since CtIP is the most upstream regulatory factor among the potential SERBP1 targets identified in this study, and the physiological outcome of SERBP1 depletion following DNA damage is similar to that of CtIP depletion. It is possible that other HR-related genes are also regulated by SERBP1 (Supplementary Figure S3B). In support of this idea, screening of HR-mediators and suppressors using the DR-GFP system indicated that proteins for RNA post-transcriptional modification including RNA processing and translation are most significantly enriched and their components also produced a strong interaction network, suggesting that regulation at the level of RNA may be involved in the expression of HR-related genes (40,41). Further elucidation of HR-related gene expression regulation at the RNA processing and translational level may provide insight into another layer of complexity in the regulatory network of HR-mediated DNA repair and cell survival strategy against DNA DSBs.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

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**REFERENCES**

1. Zhou, B.B. and Elledge, S.J. (2000) The DNA damage response: putting checkpoints in perspective. *Nature*, 408, 433–439.
2. Hakem, R. (2008) DNA-damage repair; the good, the bad, and the ugly. *EMBO J.*, 27, 589–605.
3. Jackson, S.P. and Bartek, J. (2009) The DNA-damage response in human biology and disease. *Nature*, 461, 1071–1078.
4. Chiruvella, K.K., Liang, Z. and Wilson, T.E. (2013) Repair of double-strand breaks by end joining. *Cold Spring Harbor Perspect. Biol.*, 5, a012757.
5. Decottignies,A. (2013) Alternative end-joining mechanisms: a historical perspective. Front. Genet., 4, 48.
6. Mladenov,E. and Iliakis,G. (2011) Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutat. Res., 711, 61–72.
7. Holloman,W.K. (2011) Unraveling the mechanism of BRCA2 in homologous recombination. Nat. Struct. Mol. Biol., 18, 748–754.
8. Symington,L.S. and Gautier,J. (2011) Double-strand break end resection and repair pathway choice. Annu. Rev. Genet., 45, 247–271.
9. Escribano-Diaz,C., Orthwein,A., Fradet-Turcotte,A., Xing,M., Young,J.T., Tkac,J., Cook,M.A., Rosebrock,A.P., Munro,M., Cann, M.D. et al. (2013) A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CH1P controls DNA repair pathway choice. Mol. Cell, 49, 872–883.
10. Feng,L., Fong,K.W., Wang,J., Wang,W. and Chen,J. (2013) RIF1 counteracts BRCA1-mediated end resection during DNA repair. J. Biol. Chem., 288, 11135–11143.
11. Chapman,J.R., Barral,P., Vannier,J.B., Borel,V., Steger,M., Tomas-Loba,A., Sartori,A.A., Adams,I.R., Batista,F.D. and Boulton,S.I. (2011) RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. Mol. Cell, 49, 858–871.
12. Zimmermann,M., Lottersberger,F., Buonomo,S.B., Sfeir,A. and de Lange,T. (2013) 53BP1 regulates DSB repair using Rif1 to control 5’ end resection. Mol. Cell, 57, 509–514.
13. Chen,H., Liby,M. and Symington,L.S. (2013) RPA coordinates DNA end resection and prevents formation of DNA hairpins. Molecular cell, 50, 589–600.
14. Daley,J.M. and Sung,P. (2014) 53BP1, BRCA1, and the choice between recombination and end joining at DNA double-strand breaks. Mol. Cell. Biol., 34, 1380–1388.
15. Clayton,J., Eshhar,M. and Symington,L.S. (2013) Expression and DNA end resection and prevents formation of DNA hairpins. Cell, 49, 321–329.
16. Symington,L.S. (2014) End resection at double-strand breaks: mechanism and regulation. Cold Spring Harbor Perspect. Biol., 6, a016436.
17. Longhese,M.P., Bonetti,D., Manfrini,N. and Clerici,M. (2010) Mechanisms and regulation of DNA end resection. EMBO J., 29, 2864–2874.
18. Liu,T. and Huang,J. (2014) Quality control of homologous recombination. Cell, Mol. Life Sci.: CMLS, 71, 3779–3797.
19. Sartori,A.A., Lukas,C., Coates,J., Mistrik,M., Fu,S., Bartek,J., Baer,R., Lukas,J. and Jackson,S.P. (2007) Human CtIP promotes DNA end resection. Nature, 450, 509–514.
20. Daley,J.M., Gaines,W.A., Kwon,Y. and Sung,P. (2014) Regulation of DNA pairing in homologous recombination. Cold Spring Harbor Perspect. Biol., 6, a017954.
21. Sugawara,N., Wang,X. and Haber,J.E. (2003) In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol. Cell, 12, 209–219.
22. Liu,F. and Lee,W.H. (2006) CHD activates its own and cyclin D1 promoters via the E2F/RB pathway during G1/S progression. Mol. Cell. Biol., 26, 3124–3134.
23. Heaton,J.H., Dlakic,W.M., Dlakic,M. and Gelehrter,T.D. (2001) Identification and DNA cloning of a novel RNA-binding protein that interacts with 34 nucleotides in the type-1 plasmid activation protein mRNA. J. Biol. Chem., 276, 3341–3347.
24. Costa,F.C., Saito,A., Goncalves,K.A., Vidigal,P.M., Meirelles,G.V., Bressan,G.C. and Kobarg,J. (2014) Ki-1/CDD and CDD-55 ectopic expression impact cellular pathways involved in proliferation and stress response regulation. Biochim. Biophys. Acta, 1843, 2944–2956.
25. Lemos,T.A., Passos,D.O., Nery,F.C. and Kobarg,J. (2003) Characterization of a new family of proteins that interact with the C-terminal region of the chromatin-remodeling factor CHD3. FEBS Lett., 553, 14–20.
26. Lemos,T.A. and Kobarg,J. (2006) CGI-55 interacts with nuclear proteins and co-localizes to p80-coilin positive-coiled bodies in the nucleus. Cell Biochem. Biophys., 44, 463–474.
27. Minami,O., Hainz,U., Biernacki,M.A., Zhang,W., Cai,A., Duke-Cohan,J.S., Liu,F., Brusic,V., Neuberger,D., Kutok,J.L. et al. (2010) Serologic markers of effective tumor immunity against chronic lymphocytic leukemia include nonmutated B-cell antigens. Cancer Res., 70, 1344–1355.
28. Sercer,N.B., Boesl,A., Klama,J., von Serenyi,S., Noetzel,E., Press,M.F., Dimmler,A., Hartmann,A., Schouli,J., Knuechel,R. et al. (2012) Overexpression of SERBP1 (Plasminogen activator inhibitor 1 binding protein) in human breast cancer is correlated with favourable prognosis. BMC Cancer, 12, 597.
29. Schaner,M.E., Ross,D.T., Caravagio,G., Sorlie,T., Troyanskaya,O., Diehn,M., Wang,Y.C., Duran,G.E., Sicil,T.L., Caldeira,J.S. et al. (2003) Gene expression patterns in ovarian carcinomas. Mol. Biol. Cell, 14, 4376–4386.
30. Koensgen,D., Mustea,A., Klama,J., Sun,P., Zafarakas,M., Lichtenegger,W., Denkert,C., Dahl,E. and Schouli,J. (2007) Expression analysis and DNA localization of PAI-RBP1 (SERBP1) in epithelial ovarian cancer: association with tumor progression. Gynecol. Oncol., 107, 266–273.
31. Kunkle,B.W., Yoo,C. and Roy,D. (2013) Reverse engineering of modified genes by Bayesian network analysis defines molecular determinants critical to the development of glioblastoma. PLoS One, 8, e64140.
32. Fuji,T., Dracheva,T., Player,A., Chacko,S., Clifford,R., Strausberg,R.L., Botuw,K., Azumi,N., Travis,W.D. and Jen,J. (2002) A preliminary transcriptome map of non-small cell lung cancer. Cancer Res., 62, 3340–3346.
33. Sung,W., Guo,C., Meng,X., Yu,Y., Jin,Y., Tong,D., Geng,J., Huang,Q., Qi,J., Liu,A. et al. (2012) Differential expression of PAI-RBP1, C1orf142, and COTL1 in non-small cell lung cancer cell lines with different tumor metastatic potential. J. Invest. Med., 60, 689–694.
34. Kim,M., Park,Y.J., Kim,O.K., Lee,G.Y., Chung,E.J., Sung,Y.K., Kim,J.C., Han,J. and Sohn,Y.S. (2003) Gene expression profiles related with overcoming cisplatin resistance in human cancer cell lines. Cancer Therapy, 1, 21–29.
35. Sherman-Baust,C.A., Weeraratna,A.T., Rangel,L.B., Pirzer,E.S., Cho,K.R., Schwartz,D.R., Shock,T. and Morin,P.J. (2003) Remodeling of the extracellular matrix through overexpression of collagen VI contributes to cisplatin resistance in ovarian cancer cells. Cancer Cell, 3, 377–386.
36. Lee,Y.J., Wei,H.M., Chen,L.Y. and Li,C. (2014) Localization of SERBP1 in stress granules and nucleoli. FEBS J., 281, 352–364.
37. Choi,D.W., Na,W., Kabir,M.H., Yi,E., Kwon,S., Yeon,J., Ahn,J.W., Choi,H.H., Lee,Y., Seo,K.W. et al. (2013) WIP1, a homeostatic regulator of the DNA damage response, is targeted by HIPK2 for phosphorylation and degradation. Mol. Cell, 51, 374–385.
38. Pierce,A.J., Johnson,R.D., Thompson,L.H. and Jasin,M. (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev., 13, 2633–2638.
39. Passos,D.O., Bressan,G.C., Nery,F.C. and Kobarg,J. (2009) Ki-1/57 interacts with PRMT1 and is a substrate for arginine methylation. FEBS J., 273, 3964–3961.
40. Adamson,B., Smogorzewska,A., Sigolloft,F.D., King,R.W. and Elledge,S.J. (2012) A genome-wide homologous recombination screen identifies the RNA-binding protein RBM5 as a component of the DNA damage response. Nat. Cell Biol., 14, 318–328.
41. Slabicki,M., Theis,M., Kraster,D.B., Samsonov,S., Mundwiller,E., Junqueira,M., Paszkowski-Rogacz,M., Teyra,J., Heninger,A.K., Poser,L. et al. (2010) A genome-scale DNA repair RNAi screen identifies SPG48 as a novel gene associated with hereditary spastic paraplegia. PLoS Biol., 8, e1000408.

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