ORIGINAL

Serine/arginine-rich splicing factor 7 regulates p21-dependent growth arrest in colon cancer cells

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Abstract : Serine/arginine-rich splicing factors (SRSFs) play wide-ranging roles in gene expression through post-transcriptional regulation as well as pre-mRNA splicing. SRSF7 was highly expressed in colon cancer tissues, and its knockdown inhibited cell growth in colon cancer cells (HCT116) in association with altered expression of 4,499 genes. The Ingenuity Pathway Analysis revealed that cell cycle-related canonical pathways were ranked as the highly enriched category in the affected genes. Western blotting confirmed that p21, a master regulator in cell cycle, was increased without any induction of p53 in SRSF7 knockdown cells. Furthermore, cyclin-dependent kinase 2 and retinoblastoma protein were remained in the hypophosphorylated state. In addition, the SRSF7 knockdown-induced cell growth inhibition was observed in p53-null HCT116 cells, suggesting that p53-independent pathways were involved in the SRSF7 knockdown-induced cell growth inhibition. The reduction of SRSF7 stabilized *cyclin-dependent kinase inhibitor 1A* (*CDKN1A*) mRNA without any activation of the *CDKN1A* promoter. Interestingly, SRSF7 knockdown also blocked p21 degradation. These results suggest that the reduction of SRSF7 post-transcriptionally regulates p21 induction at the multistep processes. Thus, the present findings disclose a novel, important role of SRSF7 in cell proliferation through regulating p21 levels.
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Keywords **:** *SRSF7, cell cycle, p21, G1/S arrest*

INTRODUCTION

The serine/arginine-rich splicing factor (SRSF) family, a family of essential splicing regulators, comprises 12 evolutionarily conserved and structurally related RNA-binding proteins (SRSF1-12) (31). The family members contain one or two RNA-Recognition motif (RRM) at the N-terminus and one SR domain, which mediates interaction with other proteins, at the C-terminus. Each SRSF protein was discovered as a pre-mRNA splicing factor in 1990s (14), and subsequent studies have characterized them as the multifunctional proteins in genome stability (25) and in gene expression through histone modifications (29), mRNA elongation (28), mRNA polyadenylation (30), mRNA transport (35), and translation (34).

After the discovery of the SRSF family, two classical SRSF proteins, SRSF1 (formerly SF2/ASF) and SRSF2 (formerly SC35), have been extensively investigated to uncovered crucial roles of these two proteins in cell cycle regulation, genome stability, and translation as well as pre-mRNA splicing (13). Overexpressed SRSF1 induced epithelial cell transformation through induction of alternative splicing of BLC2 family, *BIM* and *BIN1* (5). The loss of SRSF1 or SRSF2 leads formation of mutagenic R loop structure, resulting in G2/M cell cycle arrest associated with genome instability (25, 26). Reduction of SRSF3 led to the cell cycle arrest at G1 by downregulating expression of the G1-to-S checkpoint-related genes (23). A subset of SRSF proteins are overexpressed in several types of tumors, as evidenced by amplification of SRSF1 mainly in breast cancers activated by the pro-oncogenic transcription factor Myc (4, 12), and enhanced expression of SRSF3 in ovarian and colon cancers (8, 19). However, the distinct contribution of SRSF7 to cell growth or tumor progression has not been fully understood. SRSF7 was cloned in 1994 (11) and identified as an mRNA export regulator as well as a splicing factor. SRSF7 shuttles constantly between the nucleus and cytoplasm (10), and contribute to the export of certain viral and cellular mRNAs through directly binding to transporter 1 (TAP)/nuclear RNA export factor 1 (NXF1), an essential mRNA export factor (16, 20, 21, 32). In addition, overexpression of SRSF7 enhanced the association of polyribosome with a structured RNA sequence, termed the constitutive transport element (CTE), which recruits TAP/NXF1 (36).

In this study, we showed that SRSF7 knockdown induced p21 (also known as p21WAF1/CIP encoded by *cyclin-dependent kinase inhibitor 1A* (*CDKN1A*)). p21 is a well-known tumor suppressor protein, which inhibits the activity of cyclin-dependent kinase 2 (CDK2) by interfering with phosphorylation of CDK2, leading to cell-cycle arrest in response to various stimuli (1). The tumor suppressor protein p53 mediates the cell-cycle checkpoint through the activation of various growth inhibitory or apoptotic genes, including the *CDKN1A* gene. The canonical p53-p21 axis of the G1/S checkpoint pathway has a pivotal role in the DNA damage-induced transactivation of *CDKN1A* gene expression. However, post-transcriptional regulations, such as mRNA stability, translation, and proteolytic degradation, are recognized as equally important in the regulation of p21 expression.

The present study revealed that reduction of SRSF7 in colon cancer cells inhibited cell growth in association with p21 induction. The SRSF7 knockdown-induced p21 induction was due to, at least in part, increased stability of both *CDKN1A* mRNA and p21. These findings provide new insights into the role of SRSF7 in cell growth.

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MATERIALS AND METHODS

Cell culture.

HCT116 cells were cultured in Dulbecco's Modified Eagle

Medium (Nacalai Tesque, Kyoto, Japan) supplemented with 5% (vol/vol) heat-inactivated fetal bovine serum and antibiotics at 37°C in 5% CO_2.

RNA interference.

We used small interference RNAs (siRNAs) (Nippon Gene, Tokyo, Japan) targeting SRSF7 mRNA (#1, 5'-GAAAGGGCUUU-CAGUUAUU-3' ; #2, 5'-GGAUCGAGGUAUUUCCAAU-3'). A universal negative control (Nippon Gene) was used as a control siRNA. HCT116 cells were treated with the indicated siRNAs at a final concentration of 10 nM using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Quantitative real-time reverse transcription-PCR (qPCR).

Total RNA was extracted from HCT116 cells using an RNAiso plus (Takara, Otsu, Japan). One microgram of isolated RNA was reverse-transcribed using ReverTra Ace reverse transcriptase (Toyobo, Osaka, Japan). *SRSF7* and *CDKN1A* mRNA levels were measured using SYBR green master mix and the 7500 real-time system (Applied Biosystems, Foster City, CA). *Glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) and *β-actin* (*ACTB*) mRNAs were measured as internal controls for normalization. Using the ΔΔCt method, the data are presented as the fold changes in mRNA levels relative to those in control siRNA-treated cells. The following primer sets were used for *SRSF7* (forward, 5'-GCGAAGAAG-AAGCAGGTCACGGTCT-3' and reverse, 5'-TGCGTGAGCGAG-AGTATCGCCTTCC-3'), for *CDKN1A* (forward, 5'-TCACTGTCT-TGTACCCTTGTGC-3' and reverse, 5'-GGCGTTTGGAGTGGT-AGAAA-3'), for *GAPDH* (forward, 5'-AGCCACATCGCTCAGAC-AC-3' and reverse, 5'-AGCCACATCGCTCAGACAC-3'), and for *ACTB* (forward, 5'-ATTGCCGACAGGATGCAGA-3' and reverse, 5'-GAGTACTTGCGCTCAGGAGGA-3'). TissueScan tissue qPCR arrays (HCRT103) including cDNAs from paired normal and tumor tissues in 22 patients with adenocarcinomas of the colon were obtained from OriGene Technologies (Rockville, MD), and *SRSF7* levels in normal and tumor tissues were determined by qPCR. *SRSF7* levels were measured by the comparative ΔΔCt method using *ACTB* mRNA as a control and expressed as values relative to the normal samples.

Western blotting.

Whole-cell lysates were prepared in 1 x RIPA buffer (Cell Signaling Tech., Danvers, MA) containing a protease and phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). The extracted proteins were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (BioRad, Hercules, CA). After blocking with 5% non-fat dry milk, the membranes were incubated overnight at 4°C with a rabbit polyclonal anti-SRSF7 (1 : 1000 dilution ; Bethyl Laboratories, Montgomery, TX), anti-phospho-CDK2 (1 : 1000 ; Cell Signaling Tech.), anti-CDK2 (1 : 1000 ; Cell Signaling Tech.), anti-phospho-retinoblastoma protein (pRb) (1 : 1000, Cell Signaling Tech.), anti-Rb (1 : 1000, Cell Signaling Tech.), anti-p21 (1 : 2000, Santa Cruz Biotech., Santa Cruz, CA), anti-p53 (1 : 1000, Cell Signaling Tech.), or anti-GAPDH (1 : 5000 ; Santa Cruz Biotech.) antibody. The intensities of the bound antibodies were quantified by using the Image J software (NIH, Bethesda, MD).

Cell cycle analysis

Cells in S phase were assessed by counting bromodeoxyuridine (BrdU)-positive cells using an APC-BrdU Flow kit (BD pharmingen, San Diego, CA) following the manufacturer's instructions. Briefly, after treatment with the indicated siRNAs for 24 or 72 h, cells were incubated with 10 μM BrdU in the culture medium for 30 min at 37°C, harvested with Accutase (Nacalai), and washed twice with cold PBS. These cells were fixed, permeabilized by BD Cytofix/Cytoperm buffer for 15 min, and washed once with BD Perm/Wash buffer. After incubation in BD Cytoperm Plus buffer for 10 min on ice, the cells were washed again, re-fixed for 5 min at room temperature, washed once, and treated with DNase for 1 h at 37°C to expose incorporated BrdU. After washing, the cells were incubated with APC-conjugated anti-BrdU for 30 min at room temperature. The cells were incubated in staining buffer containing 7-AAD for 20 min at room temperature. They were subjected to FACS analysis. The cell cycle distribution was determined with a flow cytometer (BD FACSverse system, BD). Data were analyzed using FlowJo (Tree Star, Inc., Ashland, OR).

Reporter constructs.

For *CDKN1A* promoter activity assay, the 5' flank of the human *CDKN1A* gene was cloned into the pGL4.21 luciferase reporter vector (Promega, Madison, WI). In brief, the first PCR was performed using human genomic DNA as a template. The proximal promoter region of *CDKN1A* (from -2688 to +31 bp) was amplified using the following primer set : 5'-AAAAACTCGAGGGCTGCCT-CTGCTCAATAATGTTCT-3' (forward, XhoI site is underlined) and 5'-AAAAAAAGCTTACTGACTTCGGCAGCTGCTCACACC-3' (reverse, HindIII site is underlined). The amplified product was subcloned into the pGL4.21 vector using the XhoI and HindIII restriction sites.

Gene expression and pathway analyses.

After treatment of HCT116 cells with SRSF7 siRNA #1 or control siRNA for 48 h, total RNA was extracted as described above. After the quality of the purified RNA was assessed, gene expression was measured using a whole human genome microarray (SurePrint G3 Human GE 8 x 60K ; Agilent Technologies, Santa Clara, CA, USA) as described previously (24). Microarray data were analyzed by GeneSpring 11.5.1 (Agilent Technologies). The functional pathways related to the set of differentially expressed genes were assessed by the Ingenuity Pathway Analysis (IPA) (http : //www.ingenuity.com). The probability of a relationship between each biological function and the identified genes was calculated by Fisher's exact tests. The level of significance was set at a *P*-value of 0.05.

RESULTS

Role of SRSF7 in colon cancer cell growth.

First, we examined the expression of *SRSF7* mRNA in colorectal cancers, using 22 different cDNA libraries prepared from patients with colorectal cancers (Figure 1a). The expression levels of *SRSF7* mRNA varied depending on individual samples ; however, colorectal cancer tissues expressed significantly higher levels of *SRSF7* mRNA, compared with surrounding normal tissues.

Next, HCT116 cells were transfected with two different siRNAs (#1 and #2) targeting SRSF7, and their growth was monitored. The treatment with two different siRNAs for 48 h efficiently reduced *SRSF7* mRNA (Figure 1b) and SRSF7 protein (Figure 1c) levels. As shown in Figure 1d, both SRSF7 siRNA #1 and #2 significantly suppressed cell proliferation 48 and 72 h after their transfection. In particular, the growth was almost completely arrested with SRSF7 siRNA #1 (Figure 1d).

To determine how SRSF7 knockdown inhibited cell growth, the cell cycle status was analyzed by FACS analysis after incubation with BrdU. Since SRSF7 siRNA #1 more effectively suppressed cell growth than SRSF7 siRNA #2 did, we subjected SRSF7 siRNA #1-treated cells to FACS analysis. The percentage of BrdU-positive cells in S phase was significantly reduced to < 9% at 24 h after treatment with SRSF7 siRNA #1 compared with that of control siRNA-treated cells (around 20%). At 72 h after transfection, the percentage substantially decreased to < 2% in association with the accumulation of cells in the G0/G1 phase. These results indicated

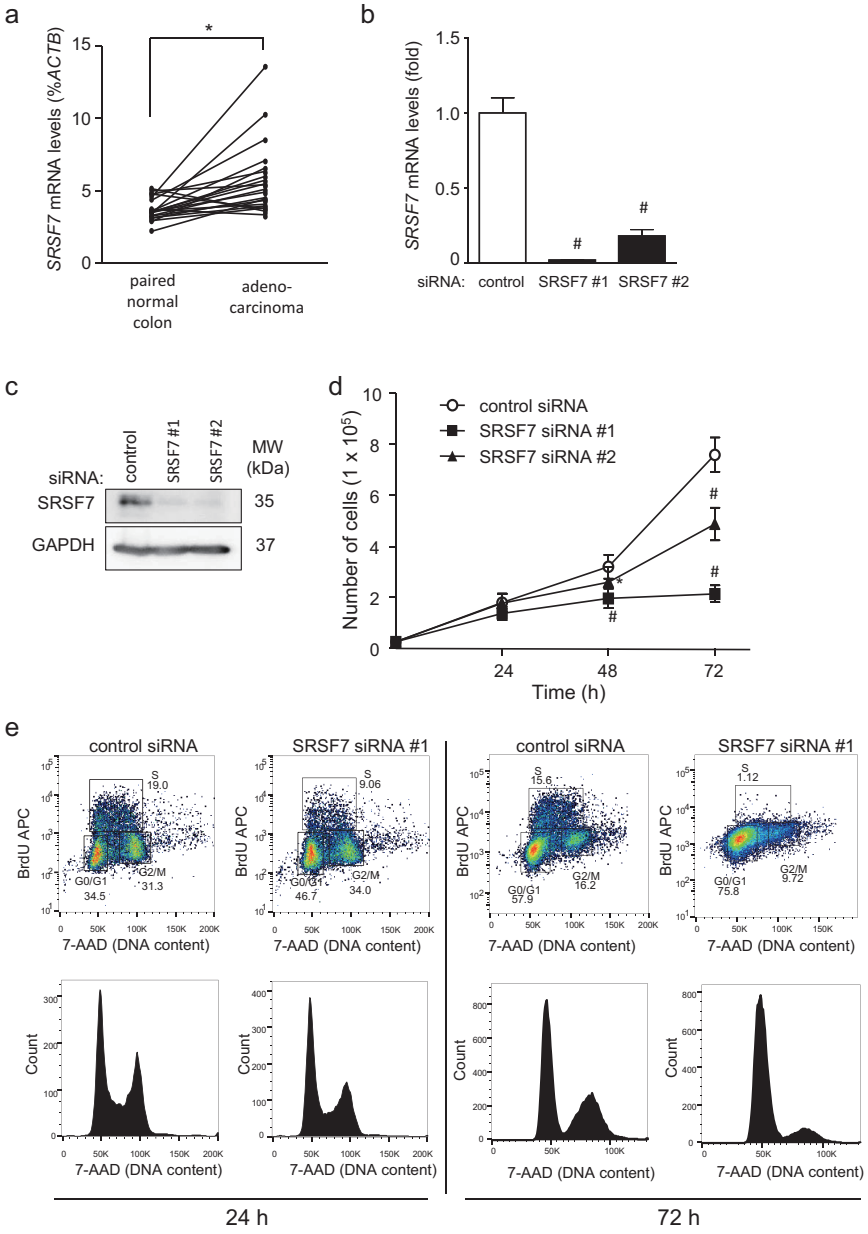

Figure 1. SRSF7 knockdown induces G1 cell cycle arrest. **(a)** *SRSF7* mRNA levels in colorectal cancer tissues and surrounding normal tissues from 22 patients were measured by qPCR using human colon cancer tissue qPCR Arrays (TissueScan, HCRT103). Values were normalized to *ACTB* mRNA levels. (*P< 0.05 by paired t-test) **(b)** After treatment with 10 nM control siRNA, SRSF7 siRNA #1 or SRSF7 siRNA #2 for 48 h, the amounts of *SRSF7* and *GAPDH* mRNAs were determined by qPCR. Values are expressed as fold changes compared with control siRNA-treated cells (mean± SD, n=5). Significantly decreased compared with control siRNA-treated cells (#P< 0.01 by unpaired Student's t-test). **(c)** Whole-cell lysates were prepared from the cells treated as described in **(b)** and subjected to Western blotting using the SRSF7 antibody. GAPDH was served as a loading control. **(d)** After treatment of HCT116 with 10 nM of SRSF7 siRNA #1 (■), #2 (▲), or control siRNA (○), the numbers of growing cells in 35-mm-diameter culture dishes were counted by a hemocytometer at 0, 24, 48 and 72 h. Values represent means±SD, n=3. (*P< 0.05 or #P< 0.01 by ANOVA and Bonferroni test) **(e)** After transfection with the indicated siRNAs for 24 or 72 h, HCT116 cells were labeled with 10 μM bromodeoxyuridine (BrdU) for 30 min at 37°C, and the percentages of BrdU-positive cells were determined by FACS analysis (upper panels). Representative histograms of cell cycle analysis of the siRNA-treated cells are shown in lower panels.

that SRSF7 knockdown blocked the cell-cycle progression from G1 to S phase.

Reduction of SRSF7 alters expression of cell cycle-related genes.

To elucidate the mechanism for the SRSF7 knockdown-induced G1 arrest, we analyzed gene expression signatures after treatment of HCT116 cells with SRSF7 siRNA #1 or control siRNA for 48 h. Microarray analysis identified 4,499 genes whose expression differed>twofold between SRSF7 knockdown and control cells ; 2,285 and 2,214 genes were down- and up-regulated in SRSF7 knockdown cells, respectively. IPA ranked "Role of BRCA1 in DNA Damage Response" (P=2E-07, z-score=-1.091), "Cell Cycle : G1/S Checkpoint Regulation" (P=9.4E-06, z-score=1.460), and "Cyclins and Cell Cycle Regulation" (P=2.25E-05, z-score=-1.877) as the top three canonical pathways related to the 4,499 affected genes (Figure 2a). Notably, IPA suggested that the activation of p53/p21 pathways likely proceeded to repress expression of their target genes including those encoding CDK2, E2F, and pRb (Figures 2a

and 2b), resulting in G1/S arrest after SRSF7 knockdown. On the other hand, the expression of other genes encoding master regulators of G1/S checkpoint (*p27*, *CDK4* and *CDK6*) was not changed in SRSF7 siRNA #1-treated cells (Figure 2b).

SRSF7 knockdown up-regulates expression of p21 in a p53-independent manner.

Western blot analysis was employed to confirm the effects of SRSF7 knockdown on the p53/p21 pathway. It should be noted that SRSF7 knockdown induced p21 without any induction of p53. p21 has a high affinity for cyclin E/CDK2 complexes (15) and prevents their activation (18). SRSF7 knockdown reduced the levels of phosphorylated CDK2 in association with decline of amounts of CDK2 protein itself. This was also in the case of pRb, a downstream target of CDK2 ; SRSF7 knockdown decreased phosphorylated pRb levels (Figure 3a).

p53 is a crucial activator of transcription of *CDKN1A* encoding p21. We therefore re-examined whether SRSF7 knockdown could p53-independently inhibit cell growth in association with the induction of p21 using p53-null ($p53^{-/-}$) HCT116 cells (kindly provided by Dr. Vogelstein). SRSF7 knockdown inhibited cell growth (Figure 3b) and induced p21 (Figure 3c), similarly as observed in wild-type HCT116 cells.

SRSF7 knockdown stabilizes CDKN1A mRNA

qPCR showed that treatment of HCT116 cells with SRSF7 siRNAs increased *CDKN1A* mRNA levels within 24 h, and its levels continued to increase at least up to 72 h (Figure 4a). To elucidate the mechanism for this increase in *CDKN1A* mRNA levels, we first examined whether the reduction of SRSF7 stimulated transcription of the *CDKN1A* gene. We cloned a luciferase reporter construct containing -2,688/+31 bp region of the human *CDKN1A* promoter and transfected it into HCT116 cells using the pGL4.21 vector. The -2,688/+31 bp region possessed the basal promoter activity of *CDKN1A* (Figure 4b). Treatment with SRSF7 siRNA #1 rather significantly suppressed the promotor activity by 30%, and SRSF7 siRNA #2 did not affect it (Figure 4b), suggesting that SRSF knockdown may not activate *CDKN1A* transcription. Next, we tested whether SRSF7 knockdown changed the degradation rate of *CDKN1A* mRNA. As shown in Figure 4c, both SRSF7 siRNA #1 and #2 significantly

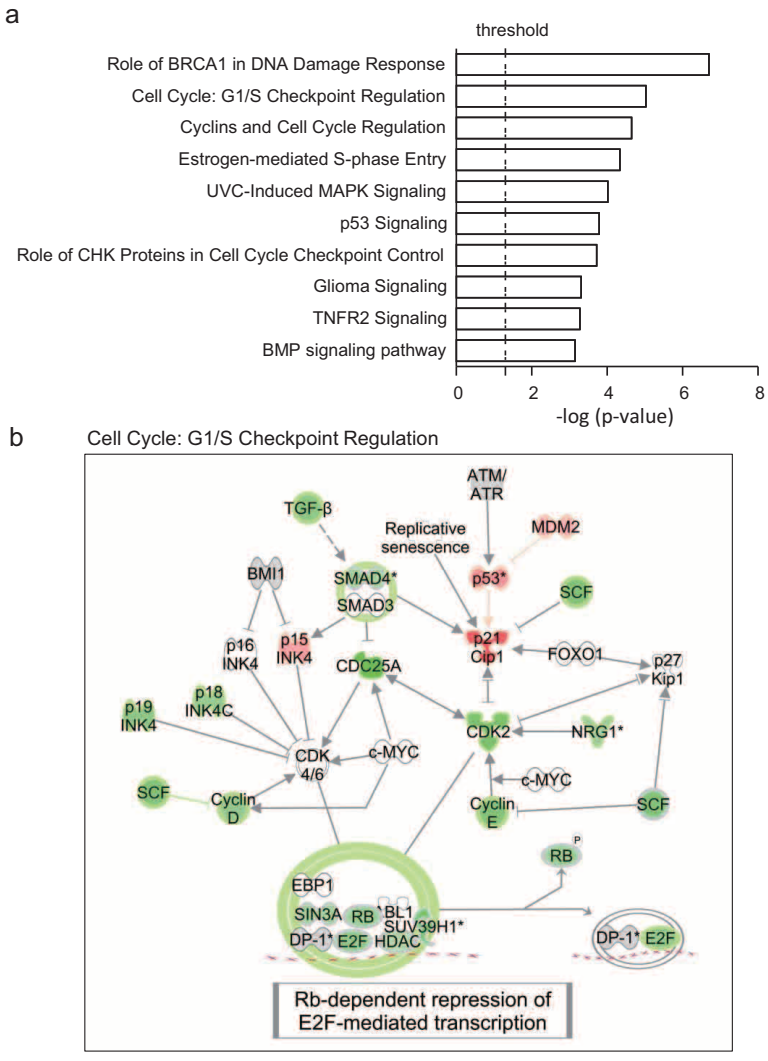

Figure 2. Cell cycle-related pathways affected by SRSF7 knockdown. **(a)** Canonical pathways affected by the 4,499 genes, whose mRNA levels were changes> twofold after SRSF7 knockdown, were analyzed using the Ingenuity Pathway analysis (IPA). The top 10 canonical pathways affected are listed. The level of significance was set at a *P* value of 0.05 (threshold) by the Fisher's exact test. **(b)** The G1/S checkpoint regulatory network represented by the differentially expressed genes is prepared using IPA. Molecules encoded by up- and down-regulated genes> twofold after SRSF7 knockdown are shown in red and green, respectively.

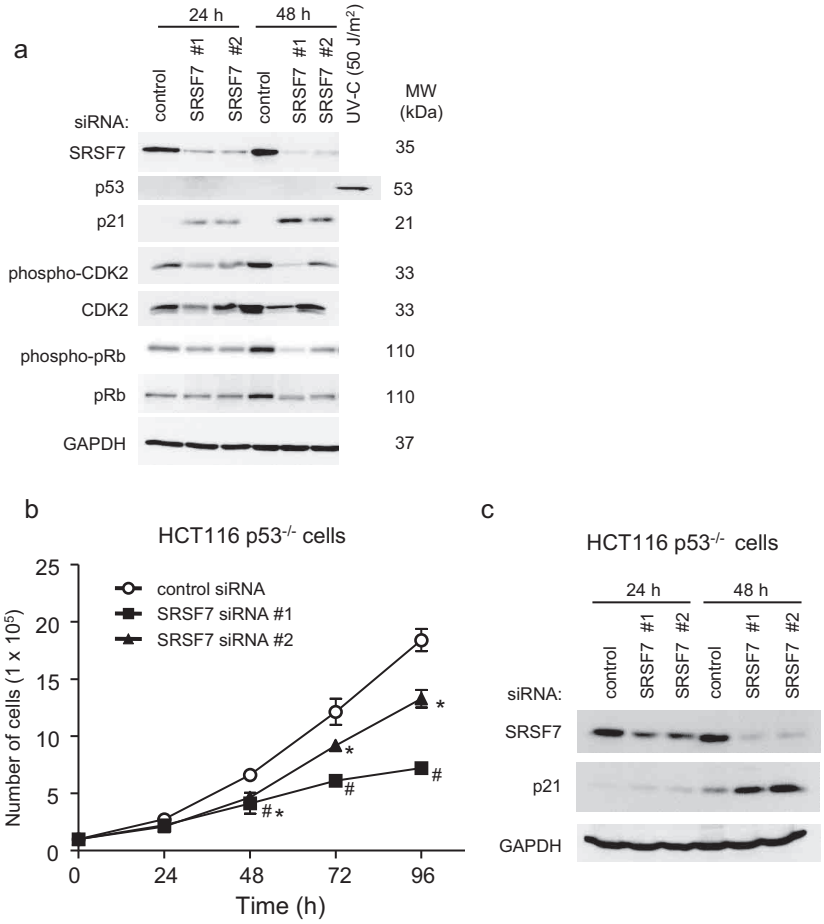

Figure 3. SRSF7 knockdown induces p21 in a p53-independent manner. **(a)** After treatment of HCT116 cells with 10 nM of the indicated siRNAs for 24 or 48 h, amounts of CDK2, phosphorylated CDK2, pRb, phosphorylated pRb, p53 and p21 in these cells were measured by Western blotting using GAPDH a loading control. The data shown are representative of three independent experiments. **(b)** After treatment of $p53^{-/-}$ HCT116 cells with 10 nM of SRSF7 siRNA #1 (■), #2 (▲), or control siRNA (○), numbers of growing cells in 35-mm-diameter culture dishes were counted by a hemocytometer at 0, 24, 48, 72 and 96 h. Values represent means ± SD, n = 6. (*$P < 0.05$ or #$P < 0.01$ by ANOVA and Bonferroni test) **(c)** After treatment of $p53^{-/-}$ HCT116 cells with 10 nM of the indicated siRNAs for 24 or 48 h, amounts of p21 in these cells were measured by Western blotting using GAPDH as a loading control. The data shown are representative of three independent experiments.

increased the stability of *CDKN1A* mRNA. These results suggest that the reduction of SRSF7 may increase p21 at least in part by stabilizing *CDKN1A* mRNA. Levels of p21 are also known to be regulated by protein degradation. We measured the stability of p21 in control- or SRSF7 siRNA #1-treated cells. In the presence of a protein synthesis inhibitor, cycloheximide, p21 was rapidly degraded in the control cells, whereas SRSF7 siRNA #1 almost completely blocked the p21 degradation during the experimental period. Thus, the reduction of SRSF7 increased p21 levels through stabilizing both its mRNA and protein.

DISCUSSION

SRSF family members are well-known regulators of both constitutive and alternative splicing reactions. We report here that SRSF7, a member of the SRSF family, plays a crucial role in the G1-to-S-phase progression through regulating p21 expression. It has been shown that the deficiency of SRSF1 (25, 26) or SRSF2 (37) facilitates R-loop formation and this genomic instability causes G2/M cell cycle arrest. On the other hand, SRSF3 knockdown induces G1 arrest in association with down-regulation of a group of genes encoding G1/S checkpoint regulators, resulting in subsequent induction of apoptosis (23). In addition, SRSF1 and SRSF3 binds histone H_3 in G2/M phase to displace heterochromatin protein 1 from mitotic chromosomes (29). Thus, each SRSF protein seems to have distinct functions in the regulation of cell fate. In contrast to SRSF1-3 examined, we could not detect any sign of DNA damage response or induction of apoptosis in SRSF7 knockdown cells. IPA analysis of the differentially expressed genes in SRSF7 knockdown cells indicated the activation of p53/p21-dependent G1 arrest pathway. However SRSF7 knockdown did not induce p53 protein. Instead, SRSF7 knockdown induced p21 not only in $p53^{+/+}$ HCT116 cells but also in $p53^{-/-}$ HCT116 cells, resulting in growth arrest of both types of cells. Thus, the reduction of SRSF7 specifically induces p21 in a p53-independent manner.

p21 belongs to the Cip/Kip family of CDK inhibitors that inhibit cell cycle progression mainly by interfering with the cyclin E/CDK2 complex (17). The kinase complex-mediated G1-to-S phase transition requires cyclin E/CDK2 and cyclin D/CDK4/CDK6, both of which cooperatively phosphorylate pRb and release E2F1 from the pRb-repressor complex (6, 7). In SRSF7 knockdown cells, CDK2 and pRb remained hypophosphorylated. *CDKN1A* mRNA was up-regulated within 12 h, and p21 levels were abundantly increased at least 24 h after treatment with SRSF7 siRNAs, suggesting that the accumulation of p21 may be crucial for the growth

inhibition in SRSF7 knockdown cells.

Although p21 expression is regulated mainly at the transcription level, several recent studies have revealed more complicated, multi-step mechanisms for the regulation of p21 expression than previously thought (1). Although SRSF7 knockdown increased *CDKN1A* mRNA levels, it rather reduced the promoter activity of *CDKN1A*. The increase in *CDKN1A* mRNA levels may be due to the increased stability of *CDKN1A* mRNA after SRSF7 knockdown. We also considered the possibility that *CDKN1A* might produce a more stable mRNA isoform through changing alternative splicing reaction. Several mRNA isoforms are transcribed from the human *CDKN1A* gene (ENSG00000124762). However only the protein coding mRNA isoform (ENST00000244741) could be detected in SRSF7 knockdown cells by RT-PCR or qPCR (data not shown).

Interestingly, compared with transcriptional or post-transcriptional regulation of *CDKN1A* mRNA expression, the stability of p21 seems to be more important in the increased expression of p21 in SRSF7 knockdown cells (Figure 4d). p21 is an unstable protein with a half-life of around 30 min. E3 ubiquitin ligase complexes (SCF^{SKP2}, $CRL4^{CDT2}$ and APC/C^{CDC20}) regulate the proteolysis of p21

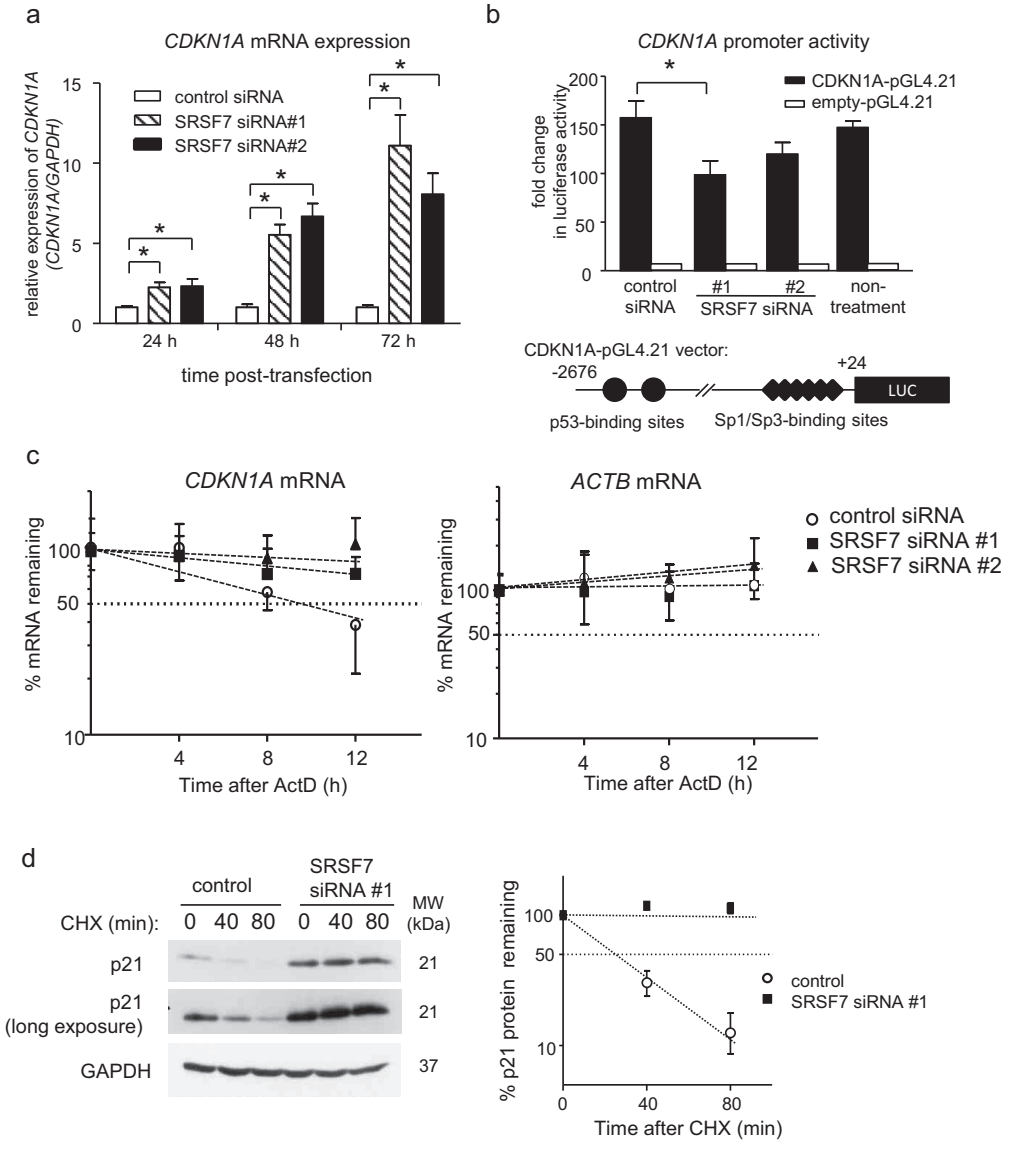

Figure 4. SRSF7 knockdown increases stability of both *CDKN1A* mRNA and p21. **(a)** After treatment of HCT116 cells with 10 nM of the indicated siRNAs for 24, 48 or 72 h, levels of *CDKN1A* mRNA were measured by qPCR using *GAPDH* mRNA as an endogenous quantity control. Values (means ± SD, n = 6) are expressed as fold changes, compared with those of control siRNA-treated cells. (*$P < 0.05$ by ANOVA and Bonferroni test) **(b)** Twenty-four hours after treatment of HCT116 cells with 10 nM *SRSF7* #1, #2 or control siRNA, these cells were transiently transfected with a luciferase reporter plasmid driven by the -2,688/+31 bp promoter fragment of *CDKN1A* (CDKN1A-pGL4.21) or a control plasmid (empty-pGL4.21) for 24 h. Luciferase activities in these cells were measured using the Dual-Luciferase Reporter Assay System. Data are presented as the fold change relative to the control plasmid transfected-cells. *Significantly decreased compared with control siRNA-treated cells ($P < 0.05$ by ANOVA and Bonferroni test). **(c)** After treatment of HCT116 cells with control siRNA (○), SRSF7 siRNA #1 (■) or SRSF7 siRNA #2 (▲) for 48 h, they were incubated in the presence of 2.5 μg/ml actinomycin D for the indicated times. Amounts of *CDKN1A* and *ACTB* mRNAs were measured by qPCR at each time point. Percentages of remaining mRNAs are plotted. **(d)** After treatment of HCT116 cells with control siRNA (○), SRSF7 siRNA #1 (■) or SRSF7 siRNA #2 (▲) for 48 h, they were incubated in the presence of 100 μg/ml cycloheximide for the indicated times. Amounts of p21 in these cells were measured by Western blotting using GAPDH as a loading control (left panel). Signal intensities of the p21 protein band are calculated using GAPDH as a loading control by ImageJ software. Percentages of remaining p21 are plotted (right panel). CHX, cycloheximide.

through the proteasome pathway (2, 3, 9, 33). Among the ligases, the gene expression analysis suggested downregulated expression of SCF^{SKP2} mRNA in SRSF7 knockdown cells. We therefore measured SKP2 levels by Western blotting. However, SKP2 levels were not changed after SRSF7 knockdown (data not shown). At present, the mechanism for the inhibition of p21 degradation remains to be elucidated.

SRSF7 was originally discovered as an mRNA export regulator (11). SRSF7 recognizes CTEs of virus mRNAs through its RRM domain, and contributes to translational activation of these mRNAs as well as mRNA export of them (20, 36). A subset of RNA-binding proteins, SRSF3 and RBM4, regulate translational activity of their targeted mRNAs through binding to 5'UTR of them (22, 27). Like these RNA-binding proteins, SRSF7 might regulate translational activity of endogenous mRNAs, including *CDKNA1A* mRNA, as well as virus mRNAs. Indeed, consensus sequences of SRSF7 binding sites are existed within 5'UTR of *CDKN1A* mRNA. It is of interest to determine whether SRSF7 regulates translational activity of p21 in SRSF7 knockdown cells. Further studies are needed to address this issue.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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