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Exon 9 skipping of apoptotic caspase-2 pre-mRNA is promoted by SRp20 through interaction with exon 8

Ha Na Jang^{#a}, Minho Lee^{#a}, Tiing Jen Loh^a, Seung-Woo Choi^a, Hyun Kyung Oh^a, Heegyum Moon^a, Sunghye Cho^a, Seong-Eui Hong^a, Do Han Kim^a, Zhi Sheng^b, Michael R Green^c, Daeho Park^a, Xuexiu Zheng^a, and Haihong Shen^{a,*}

^aSchool of life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea.

^bVirginia Tech Carilion Research Institute, Roanoke, VA, 24016, USA; Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 24061, USA

^cHoward Hughes Medical Institute and Programs in Gene Function and Expression and Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA.

[#] These authors contributed equally to this work.

Abstract

Alternative splicing plays an important role in gene expression by producing different proteins from a gene. Caspase-2 pre-mRNA produces anti-apoptotic Casp-2S and proapoptotic Casp-2L proteins through exon 9 inclusion or skipping. However, the molecular mechanisms of exon 9 splicing are not well understood. Here we show that knockdown of SRp20 with siRNA induced significant increase of endogenous exon 9 inclusion. In addition, overexpression of SRp20 promoted exon 9 skipping. Thus we conclude that SRp20 promotes exon 9 skipping. In order to understand the functional target of SRp20 on caspase-2 premRNA, we performed substitution and deletion mutagenesis on the potential SRp20 binding sites that were predicted from previous reports. We demonstrate that substitution mutagenesis of the potential SRp20 binding site on exon 8 severely disrupted the effects of SRp20 on exon 9 skipping. Furthermore, with the approach of RNA pulldown and immunoblotting analysis we show that SRp20 interacts with the potential SRp20 binding RNA sequence on exon 8 but not with the mutant RNA sequence. In addition, we show that a deletion of 26 nt RNA from 5' end of exon 8, a 33 nt RNA from 3' end of exon 10 and a 2225 nt RNA from intron 9 did not compromise the function of SRp20 on exon 9 splicing. Therefore we conclude that SRp20 promotes exon 9 skipping of caspase-2 pre-mRNA by interacting with exon 8. Our results reveal a novel mechanism of Caspase-2 pre-mRNA splicing.

*Corresponding author at: Gwangju Institute of Science and Technology, Life Sciences, 123, Cheomdan Gwagiro, Buk-gu, Gwangju, Korea, Republic of. Fax: 82-62-715-2484. haihongshen@gist.ac.kr.

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Keywords

Caspase-2; SRp20; Apoptotic; anti-apoptotic; pre-mRNA splicing; exon 9

1. Introduction

Pre-mRNA splicing plays an essential role in the gene expression of higher eukaryotes [1]. The process of intron removing occurs in a large RNA-protein complex called spliceosome. Spliceosome is assembled by forming prespliceosome (complex A) first, subsequently forming mature and catalytically active (complex B and C) spliceosome [2, 3]. Pre-mRNA splicing reaction is divided into two consecutive steps: in the first step, the 5' splice site is cleaved and a lariat structure of intron and the second exon is formed; in the second step, the 3' splice site is cleaved and two exons are ligated each other [4]. Alternative splicing produces various protein isoforms with different biological functions from a gene [5, 6]. More than 90% of human genes are alternatively spliced [7, 8].

Apoptosis is the process of programmed cell death, plays a fundamental role in embryonic development and homeostasis [9]. It was shown that alternative splicing regulates apoptosis by producing pro-apoptotic and anti-apoptotic isoforms from one pre-mRNA, as shown in Bcl-x, Fas and Caspase-2 pre-mRNAs [10, 11]. Anti-apoptotic pathways are essential for tumorigenesis and the resistance to cancer drugs. Caspase-2 is one of the initiator caspases which are activated in the apoptosis process [12]. Two discrete isoforms are produced from Caspase-2 pre-mRNA through alternative splicing [13]. Exon 9 skipping of caspase-2 produces a pro-apoptotic Casp-2L protein, which includes an active domain of the enzyme. By contrast, exon 9 inclusion of caspase-2 leads to the production of an in-frame stop codon at exon 10, thus an anti-apoptotic Casp-2S protein with lack of the enzyme active domain is made (figure 1A)[14-16]. It was shown previously that an In100 element downstream of intron 9 inhibits exon 9 inclusion by acting as a "decoy" splicing acceptor [17]. In addition, RBM5 promotes exon 9 inclusion through binding to the U/C rich intronic sequence immediately upstream of In100 [18]. Furthermore, SC35 and hnRNP A1 were shown to promote or inhibit exon 9 skipping of Caspase-2 pre-mRNA [19]. However, the alternative splicing mechanism of exon 9 is largely unknown.

SRp20 is a member of SR (Serine-Arginine rich) protein family, a group of proteins essential for general splicing as well as alternative splicing [20-23]. SRp20 has been shown to regulate alternative splicing of CD44, Tau and Fibronectin pre-mRNA [24-26]. SRp20 is also essential in RNA polyadenylation, RNA export and protein translation [27-30]. Knockdown of SRp20 causes apoptosis in ovarian cancer cells and its expression is associated with malignancy of epithelial ovarian cancer [31]. SRp20 promotes tumor induction and the maintenance of tumor growth in nude mice and renders immortal rodent fibroblasts tumorigenic [32].

In this study, we show that SRp20 promotes exon 9 skipping of caspase-2 pre-mRNA. We found that knockdown of SRp20 promotes exon 9 inclusion of caspase-2 pre-mRNA, whereas overexpression of SRp20 promotes exon 9 skipping. Thus we conclude that SRp20

promotes exon 9 skipping. Furthermore, we demonstrate that SRp20 functions through interacting with exon 8 to promote exon 9 skipping.

2. Materials and methods

2.1. Construction of plasmids

All primers and oligonucleotides for plasmid construction are listed in table 1. Caspase-2 minigene constructs were made by inserting the human Caspase-2 genomic fragment into pCI-neo plasmid using restriction enzymes NheI (Takara) and XhoI (Takara). Specifically, DNA containing exon 8-10 of Caspase-2 was PCR-amplified with primer sets (forward: E8E10F, reverse: E8E10R) using human genomic DNA as a template. To generate mutant constructs Del-exon, Del-exon-intron and SRp20mut, we performed overlapping PCR reactions. Following primers were used for different constructs: primers for Del-exon constructs (forward: E8-26ntF, reverse: E10-33R], primers for Del-exon-intron construct (forward: I9-2225F, reverse: I9-2225R], primers for SRp20mut construct (forward: SRp20F, reverse: SRp20R).

2.2 Cell culture and plasmid transfection

HeLa and HEK293T cells were cultured at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM Glutamine, 100 IU/ml penicillin and 100 ug/ml streptomycin. MDA MB 231 cells were grown in Roswell Park Memorial Institute Medium (RPMI). Plasmid transfection was performed using polyethyleneimine (PEI) (Sigma) reagent. Plasmid was mixed with PEI in 100 ul FBS free-media. The mixtures were added to cells in 900 ul of pre-warmed media supplemented with FBS. The cells were incubated for 5 h and then replaced with 2 ml of fresh complete growth medium. RNA was extracted after 48 h transfection.

2.3. RT-PCR

RNA were extracted from cells using RiboEX reagent (GeneAll) following the manufacturer's instructions. cDNA for RT-PCR was reverse transcribed from 1 ug of total RNA using oligo-dT₁₈ primer and ImProm-II™ reverse transcriptase (Promega). One microliter of cDNA was used for PCR and loaded onto 2% agarose gels or 5% polyacrylamide gels and visualized with by staining with ethidium bromide solution (0.5 ug/ml). Following primers were used for RT-PCR analysis: primers for endogenous Caspase-2 exon 8-10 (forward: endoF, reverse: endoR), primers for exogenous caspase-2 minigenes (forward: exoF, reverse: exoR), primer for detecting SRp20 mRNA (forward: SRp20RTF, reverse: SRp20RTR), primers for detecting GAPDH mRNA (forward: GAPDHF, reverse: GAPDHR).

2.4. shRNA treatment

293T cells (1×10^6 cells on a 35 mm plate) were transfected with psPAX2 (the packaging vector), pMD2.G (the envelope vector) and shRNA plasmid (Open Biosystems) DNA with 0.67 ug/ml polyethyleneimine (PEI) (Sigma) reagent in 1.8 ml FBS free-DMEM. After 48 h, the supernatant containing viral particles was filtered through a 0.45 um filter. HeLa and

MDA MB 231 cells were infected with either non-silencing shRNA or SRp20 shRNA with 5 mg/ml polybrene (Sigma) treatment. Total RNAs were extracted after 72 h.

2.5. RNA binding assay of SRp20

RNA binding analysis of SRp20 were performed as described previously [33]. Briefly, chemically synthesized 5' biotin labeled wild type (UUCUUCAUCC) and mutant (UGAAGCGUCC) RNA were covalently linked with Streptavidin agarose conjugate (Millipore). Streptavidin agarose linked biotin-RNA was incubated with HeLa nuclear extract for 3.5 h at 4 °C in buffer D (20mM Tris-Cl, 100mM KCl, 2mM EDTA, 20% glycerol, 0.5mM DTT, 0.5mM PMSF, pH 7.5). After washing, SDS PAGE loading buffer were added and boiled. Supernatants were analyzed by immunoblotting analysis with SRp20 antibody.

2.6. Immunoblotting analysis

Cells were lysed by incubating with lysis buffer (0.1 % triton X-100, 50 mM Tris-Cl pH7.5, 150 mM NaCl, 5 mM EDTA, 1 mM beta-mercaptoethanol) for 1 h at 4 °C. The supernatant was collected after centrifuge and then used for immunoblotting analysis. The following primary antibodies were used: mouse monoclonal anti-SRp20 (1:1,000; Zymed Laboratory).

3. Results

3.1. Reduced expression of SRp20 promotes exon 9 inclusion of caspase-2 pre-mRNA

In order to determine the pre-mRNA splicing events of caspase-2, we performed RT-PCR analysis with a primer pair which basepair with exon 8 and 10 separately (figure 1A). As shown in figure 1B, exon 9 skipped mRNA isoform is predominantly expressed in both MDA MB 231 and HeLa cells (~90% and ~93%), whereas exon 9 included mRNA isoform is expressed at much lower level (~10% and ~7%).

By analyzing the sequence of caspase-2 pre-mRNA, we found that exon 8-10 includes a number of potential binding sites for SRp20 [(C/U)(A/C/U)(U/A)(C/A/U)(A/C/U)] [34]. Thus we considered the possibility that SRp20 regulates caspase-2 pre-mRNA splicing. In order to determine the role of SRp20 in the pre-mRNA splicing of caspase-2, we treated MDA MB 231 cells with shRNA virus that targets SRp20 mRNA. We extracted RNA and then performed RT-PCR analysis for exon 9 splicing of endogenous Caspase-2. As shown in figure 2A, SRp20 expression is reduced significantly compared with uninfected cells, as shown with RT-PCR analysis for SRp20 mRNA and immunoblotting analysis with anti-SRp20 antibody, with GAPDH and α -tubulin as the loading controls independently (lane 3, lower panel). By contrast, non-silencing shRNA virus did not induce significant decrease of SRp20 expression (lane 2). We demonstrate that knockdown of SRp20 promoted significant increase of exon 9 inclusion isoform (~16%) (lane 3, upper panel)(figure 2A). However non-silencing siRNA treatment did not cause significant alteration of exon 9 inclusion. Thus we conclude that knockdown of SRp20 induced the increase of exon 9 inclusion in MDA MB 231 cells. In order to ask if the effects of SRp20 are specific to MDA MB 231 cells or not, we performed the shRNA knockdown experiments on HeLa cells. As shown in figure 2B, consistent with the results of figure 2A, Knockdown of SRp20 promoted the increase of

exon 9 inclusion significantly in MDA MB 231 cells (~12%). Therefore we conclude that reduced expression of SRp20 promotes exon 9 inclusion of caspase-2 pre-mRNA both in MDA MB 231 and HeLa cells.

3.2. Expression of SRp20 promoted exon 9 skipping rate of caspase-2 pre-mRNA

We next asked if SRp20 overexpression has the opposite effects on caspase-2 pre-mRNA splicing as compared with the SRp20 knockdown effects. To answer the question, we used a minigene that includes genomic DNA of caspase-2 exon 8-10 (figure 3A). We first overexpressed SRp20 protein along with caspase-2 minigene, then performed RT-PCR analysis for exon 9 splicing using primers shown in figure 3A, one of which basepair with exon 8, the other one of which basepair with the plasmid, as shown with arrows in figure 3A. As a positive control, we overexpressed SC35 expression plasmid along with the caspase-2 minigene. The results in figure 3B (left panel) show that SRp20 expression induced significant decrease of exon 9 inclusion rate (~19%) in MDA MB 231 cells (lane 3), whereas expression of control plasmid did not (lane 2). Consistent with previous results, SC35 promotes exon 9 skipping rate significantly (~37%) (figure 3B, right panel)[19]. Thus we conclude that SRp20 expression promotes exon 9 skipping rate of caspase-2 pre-mRNA. We next asked whether the effects of SRp20 on Caspase-2 pre-mRNA slicing are specific to MDA MB 231 cells. To answer the question, we expressed SRp20 plasmid in HeLa cells along with the Caspase-2 minigene. Figure 2C shows that SRp20 also promotes exon 9 skipping rate significantly (~22%) in MDA MB 231 cells. Similarly, SC35 promotes exon 9 skipping rate significantly in HeLa cells (~38%) (figure 3B, right panel) [19]. In combination with the results of figure 2, we conclude that SRp20 promotes exon 9 skipping rate of caspase-2 pre-mRNA.

3.3. A 26 nt RNA at 5' end of exon 8, a 33 nt RNA at 3' end of exon 10 and a 2225 nt RNA at middle of intron 9 are not responsible for the function of SRp20 on exon 9 splicing

In order to determine the SRp20 responsive elements on caspase-2 pre-mRNA, we performed mutagenesis analysis. Among the RNA sequences that include potential binding sites for SRp20, We first asked if the 3' region of exon 8 and 5' region of exon 9 affects the function of SRp20. Thus we deleted 26 nt RNA and 33 nt RNA from exon 8 and exon 10 respectively (Del-exon) (figure 4A). We transfected the deletion mutant minigene plasmid into HeLa cells, extracted RNA and then performed RT-PCR analysis. If the deleted RNA is responsible for the function of SRp20, We expect that exon 9 skipping rate is decreased for the deletion mutant; in addition, SRp20 would not promote the skipping rate of the deletion mutant minigene. In contrary to our prediction, RT-PCR results of Del-exon minigene show that SRp20 promotes exon 9 skipping rate significantly both in HeLa and MDA MB 231 cells (~10% and ~12% independently) (lanes 3 and 6, figure 4B). Therefore we conclude that SRp20 does not function through the 26 nt RNA at 5' end of exon 8 and the 33 nt RNA at 3' end of exon 10.

We noticed that the middle part of intron 9 includes seven potential SRp20 binding sites (not shown). Thus we next asked if the intron 9 RNA is responsible for the function of SRp20. In order to address the question, we deleted a 2225 nt intron 9 RNA, which is located at 565 nt downstream from the 3' splice site of exon 9, from Del-exon minigene to construct another

minigene (Del-exon-intron) (figure 4A). Figure 4C show that exon 9 inclusion rate was not altered significantly in Del-exon-intron minigene (lanes 1 and 4, figure 4C). In addition, overexpression of SRp20 still induced significant increase of the exon 9 splicing of caspase-2 in the Del-exon-intron minigene (~12%) (lane 3, figure 4C). Similar effects of SRp20 on exon 9 splicing was observed in MDA MB 231 cells (~17%) (lane 6, figure 4C). Thus the 2225 nt RNA in intron 9 is not required for the function of SRp20. Taken together, we concluded that SRp20 does not function through a 26 nt RNA at 3' end of exon 8, a 33 nt RNA at 5' end of exon 10 or a 2225 nt RNA at middle of intron 9 in the promoting of exon 9 skipping.

3.4. SRp20 functions through interacting with exon 8

We noticed that exon 8 contains a potential SRp20 binding site (UCUUCAUC), which is located at 24 nt upstream from the 5' splice site of exon 8 (figure 5A) [34-37]. We asked if this potential SRp20 binding site functions as the target of SRp20 to regulate exon 9 splicing. We hypothesize that SRp20 contact the potential binding site on exon 8 to make the 5' splice site of exon 8 relatively stronger than the 5' splice site of exon 9, leading to exon 9 skipping of caspase-2 pre-mRNA. In order to test this idea, we performed mutagenesis analysis. As shown in figure 5A, we mutated the strong SRp20 binding site (UCUUCAUC) into GAAGCGUC to compromise the potential binding of SRp20 (SRp20mut) (figure 5A). If the sequence on exon 8 is responsible for the function of SRp20, we expect that exon 9 inclusion of the SRp20mut minigene would be decrease, which is similar to that of SRp20 knockdown effects. In addition, the effects of SRp20 on exon 9 splicing of caspase-2 will be largely disrupted, as its functional target is compromised. As we predicted, exon 9 inclusion was decreased in the mutant minigene significantly (compare lane 1, 4 of figure 5 with lane 1, 4 of figure 4). Furthermore, SRp20 did not promote exon 9 skipping in the SRp20mut minigene (~0%) (lane 6, figure 5B). Thus we conclude that SRp20 functions through the potential binding site of SRp20 on exon 8. We next asked whether SRp20 functions by directly binding to the potential binding site on exon 8. To answer the question, we synthesized 5' biotin labeled 10 nt RNA of wild type (UUCUUCAUCC) and, as a control, a 10 nt SRp20mut RNA sequence (UGAAGCGUCC). After incubation of the biotin-labeled RNA with HeLa nuclear extract, we precipitated RNA binding proteins with streptavidin bead, performed immunoblotting analysis using anti-SRp20 antibody. As shown in figure 5C, SRp20 contacts the potential binding site on exon 8. However, the binding of SRp20 was completely abolished for the SRp20mut RNA. Therefore we conclude that SRp20 promotes exon 9 skipping through interacting with exon 8.

4. Discussion

In this paper, we studied the basis by which exon 9 of Caspase-2 pre-mRNA is spliced. We demonstrate here that knockdown of SRp20 induces exon 9 inclusion significantly, whereas overexpression of SRp20 promotes exon 9 skipping. Thus we conclude that SRp20 promotes exon 9 skipping. In order to identify the functional targets of SRp20, we performed substitution and deletion mutagenesis analysis. Our results demonstrate that a disruption of the potential SRp20 binding site on exon 8 abolished the function of SRp20 on exon 9

skipping. Furthermore, using RNA pulldown and immunoprecipitation analysis, we show that SRp20 interacts with the potential binding site on exon 8. In addition, we found that a deletion of 26 nt RNA from 3' end of exon 8, a 33 nt RNA from 5' end of exon 10 or a 2225 nt RNA from intron 9 did not compromise the function of SRp20 on exon 9 splicing. Our results provide a novel mechanism by which Caspase-2 exon 9 is spliced.

4.1 SRp20 promotes exon 9 skipping of Caspase-2 pre-mRNA

It is well known that SR protein family promotes splicing by activating spliceosome recruitment. SRp20 was shown to promote exon inclusion in some cases [24], however it was also shown that SRp20 promotes exon skipping [25]. Our results demonstrate that SRp20 promotes exon 9 skipping of Caspase-2 pre-mRNA through interacting with exon 8. Previously it was shown that exon 9 splicing of Caspase-2 pre-mRNA is regulated through multiple RNA proteins such as SC35 and hnRNP A1 [10]. Our results here added SRp20 as another regulator for exon 9 splicing of Caspase-2 pre-mRNA. Our results suggest that SRp20 promotes proximal splice site selection, furthermore, inhibits distal splice site selection, and consequently promotes exon 9 skipping of caspase-2 pre-mRNA. We have previously reported that hnRNP A1 promotes exon 6 inclusion of Fas pre-mRNA by contacting exon 5 [33]. The results indicate that hnRNP A1, opposite to SRp20, promotes distal splice site selection, inhibits proximal splice site selection, promotes exon 6 inclusion. Taken together, the effects of SR proteins or hnRNP proteins on alternative splicing are determined by the binding positions of the regulatory proteins on pre-mRNA.

4.2 A proximal potential SRp20 binding site from splice sites is the Functional target

Based on the previous reports, we predicted that a number of potential SRp20 binding sites on the caspase-2 pre-mRNA [34-37]. Among them, we demonstrate that a potential SRp20 binding sequence on exon 8, which is the closest one among all of the potential binding sequence of SRp20. Previous reports were based on the results of SELEX experiments. It is not surprising that the prediction results would provide multiple potential binding sites. Thus it would be difficult to test the function of all of the potential binding sites. Our previous report [33] and our results here demonstrate that potential binding sites located close to splice site are the functional targets. Therefore it will be much convenient to analyze the potential sites that close to splice sites and then localize the functional targets of regulatory proteins on pre-mRNA.

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Highlights

- SRp20 promotes exon 9 skipping caspase-2 pre-mRNA.
- SRp20 interacts with exon 8 to regulate exon 9 splicing.
- 5' end of exon 8, 3' end of exon 10 or middle intron 9 are not targets of SRp20.

Figure 1.

(A) Alternative splicing of caspase exon 9 is shown. Exon 9 inclusion creates a stop codon at exon 10 to produces an anti-apoptotic (Casp-2S) protein; whereas exon 9 skipping produces a pro-apoptotic (Casp-2L) protein. The functional domains of caspase-2 protein are shown at bottom. (B) RT-PCR analysis of Caspase-2 exon 9 splicing in HeLa and MDA MB 231 cells (left panel). The quantitation results for exon 9 skipping of total RNA are shown (right panel).

Figure 2.

Reduced expression of SRp20 promotes endogenous exon 9 inclusion of caspase-2 pre-mRNA. RT-PCR analysis for endogenous Caspase-2 exon 9 inclusion/skipping was performed with RNAs from non-treated cells, non-silencing shRNA virus treated cells and SRp20 shRNA virus treated cells. Primers used for the RT-PCR reaction are basepaired with exon 8 and 10 as shown with arrows. Expression of SRp20 is demonstrated with RT-PCR and immunoblotting analysis, GAPDH and α -tubulin were used as loading controls independently. Quantitation analysis for exon 9 inclusion of total RNA is shown (right panel). HeLa cells (A) and MDA MB 231 cells (B) were analyzed.

Figure 3.

Expression of SRp20 promoted exon 9 skipping of caspase-2 pre-mRNA. (A) A minigene of caspase 2 includes exon 8-10. Primers used for RT-PCR for exon 9 splicing are shown with arrows. (B) RT-PCR analysis of exon 9 inclusion for the minigene was performed with cells overexpressing pcDNA-SRp20 plasmid, pcDNA plasmid and without treatment. The quantitation results of exon 9 inclusion of total RNA are shown at right panel. HeLa (A) and MDA MB 231 cells (B) were used in the analysis.

Figure 4.

A 26 nt RNA at 3' end of exon 8, a 33 nt RNA at 5' end of exon 10 and a 2225 nt RNA at middle of intron 9 are not responsible for the function of SRp20 on exon 9 splicing. (A) Wild type and deletion mutant minigenes are shown. In the Del-exon minigene, a 26 nt RNA at 3' end of exon 8 and a 33 nt RNA at 5' end of exon 10 are deleted. In the Del-exon-intron minigene, a 26 nt RNA at 3' end of exon 8, a 33 nt RNA at 5' end of exon 10 and a 2225 nt RNA at middle of intron 9 are deleted. The positions of deleted RNAs are shown. The primers that were used for RT-PCR analysis are shown with arrows. (B) RT-PCR analysis of exon 9 splicing for Del-exon minigene in the cells with expression of pcDNA-SRp20 plasmid. Untreated cells and pcDNA plasmid expressing plasmid were used as loading controls. HeLa cells and MDA MB 231 cells were used. The quantitation of exon 9 inclusion of total RNA is shown at the bottom panel. (C) RT-PCR analysis of exon 9 splicing for Del-exon-intron minigene in the cells with expression of pcDNA-SRp20 plasmid. HeLa cells and MDA MB 231 cells were used. The quantitation of exon 9 inclusion of total RNA is shown at the bottom panel.

Figure 5.

SRp20 functions through interacting with exon 8. (A) The location and sequence of SRp20 binding sites on exon 8 are shown (wild type). The sequence of mutated SRp20 binding site in the mutant minigene (SRp20mut) is also shown. (B) RT-PCR analysis of exon 9 inclusion in the SRp20mut minigene was performed with cells overexpressing pcDNASRp20, pcDNA plasmid and without treatment. The quantitation results of exon 9 inclusion of total RNA are shown at lower panel. HeLa and MDA MB 231 cells were used in the analysis. (C) RNA pulldown and immunoblotting analysis with anti-SRp20 antibody was shown with the 10 nt potential SRp20 binding site RNA and a mutant RNA (SRp20mut). The sequences of wild type and SRp20mut are shown. RNAs are labeled at 5' end.

Table 1

Primer list

Name	Sequence
E8E10F	5'-CTAGCTAGCCACCATGCTCCAAGAGGTTTTTCAGCTC-3'
E8E10R	5'-CCGCTCGAGCCCCCCCCCCCCCTTTGAGGCAGGCATAGCC-3'
E8-26F	5'-CTAGCTAGCCACCATGGCCAACGCCAACTGCCCAAG-3'
E10-33R	5'-CCGCTCGAGCCCACCTCCACTAATAAGCGCGTGGGCAGTCTC-3'
I9-2225F	5'-GACTTCACCATTTACAACCACCTTGGAAC-3'
I9-2225R	5'-GTTTCCAAGGTGGTTGTAAATGGTGAAGTC-3'
SRp20F	5'-CCAAAAATGTGAAGCGTCCAGGCCTGCC-3'
SRp20R	5'-GGCAGGCCTGGACGCTTCACATTTTGG-3'
endoF	5'-CCCAAGCCTACAGAACAAACC-3'
endoR	5'-CTTTACCGGCATCACTCTCC-3'
exoF	5'-CCCAAGCCTACAGAACAAACC-3'
exoR	5'-CTG AAG CAC TGC ACG CCG TAG-3'
SRp20RTF	5'-AACAAGACGGAATTGGAACGG-3'
SRp20RTR	5'-GTGGGCCACGATTCTACTTC-3'
GAPDHF	5'-ACCACAGTCCATGCCATCA-3'
GAPDHR	5'-TCCACCACCCTGTTGCTGTA-3'