BMB
Reports

BMB Rep. 2019; 52(11): 641-646
www.bmbreports.org

RRM but not the Asp/Glu domain of hnRNP C_1/C_2 is required for splicing regulation of Ron exon 11 pre-mRNA

Heegyum Moon[1, †], Ha Na Jang[1], Yongchao Liu[1], Namjeong Choi[1], Jagyeong Oh[1], Jiyeon Ha[1], Hyeon Ho Kim[2], Xuexiu Zheng[1,] & Haihong Shen[1,*]*

[1]School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 61005, [2]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences and Technology, Sungkyunkwan University, Seoul 06351, Korea

The Ron proto-oncogene is a human receptor for macrophage-stimulating protein (MSP). The exclusion of exon 11 in alternative splicing generates ΔRON protein that is constitutively activated. Heterogenous ribonucleaoprotein (hnRNP) C_1/C_2 is one of the most abundant proteins in cells. In this manuscript, we showed that both hnRNP C_1 and C_2 promoted exon 11 inclusion of Ron pre-mRNA and that hnRNP C_1 and hnRNP C_2 functioned independently but not cooperatively. Moreover, hnRNP C_1 stimulated exon 11 splicing through intron 10 activation but not through intron 11 splicing. Furthermore, we showed that, whereas the RRM domain was required for hnRNP C_1 function, the Asp/Glu domain was not. In conclusion, hnRNP C_1/C_2 promoted exon 11 splicing independently by stimulating intron 10 splicing through RRM but not through the Asp/Glu domain. [BMB Reports 2019; 52(11): 641-646]

INTRODUCTION

Pre-mRNA splicing occurs in a large RNA-protein complex called a spliceosome (1). Spliceosome assembly is a stepwise process in which U1 snRNP basepairs with a 5′ splice-site, then U2 snRNP basepairs with a branch-point. Then, the U4/U5/U6 snRNP is loaded into the complex. Through alternative exon inclusion/exclusion, proteins with slightly different, completely different, and opposite functions are produced (2-4).

Ron is a cell surface-located receptor composed of disulfide-linked α and β subunits, which are produced from a 190 kDa single chain precursor by proteolytic cleavage (5). ΔRON is an uncleaved protein isoform lacking 49 amino acids in the extracellular domain, which is produced by the exclusion of exon 11 in alternative spicing procedures (6). For ΔRON is unable to bind the ligand, it must be constitutively activated by tyrosine phosphorylation through intracellular oligomerization (6, 7). SRSF1, SRSF2, and hnRNP A1 have been demonstrated to regulate exon 11 splicing (8-10).

Heterogeneous nuclear ribonucleoprotein (hnRNP) C_1/C_2 is one of the most abundant proteins in cells (11). hnRNP C_1/C_2 includes an N-terminal RNA recognition motif (RRM), a basic leucine zipper (bZIP)-like motif (bZLM), and an acidic aspartic acid/glutamic acid-rich (Asp/Glu) domain (UniprotKB - P07910) (12). While RRM plays a minimal role in the overall affinity of hnRNP C_1/C_2 for RNA, a highly basic 40 amino acid (aa) domain preceding the leucine zipper motif provides high-affinity for RNA (13, 14). An acidic Asp/Glu domain is necessary for homo- or heterotetramer formation of hnRNP C_1 and C_2 (13). hnRNP C_1/C_2 inhibits splicing of the Alu element in the human genome by interfering with $U2AF^{65}$ binding to the cryptic PPT sequence (15).

Here, we show that hnRNP C_1/C_2 promoted exon 11 inclusion of Ron pre-mRNA through stimulation of intron 10, but not intron 11, splicing. In addition, we found that hnRNP C_1 and hnRNP C_2 functioned independently, not cooperatively. Importantly, although the RRM domain was required for hnRNP C_1/C_2 function, the Asp/Glu domain of hnRNP C_1 was not necessary for promoting Ron exon 11 splicing.

RESULTS

Reduced hnRNP C_1/C_2 expression inhibits exon 11 splicing of Ron pre-mRNA

In order to understand the role of hnRNP C_1/C_2 in the splicing of Ron pre-mRNA, we investigated whether reduced expression of hnRNP C_1/C_2 affected Ron alternative splicing. Fig. 1A shows that hnRNP C_1/C_2-targeted shRNA treatment reduced both hnRNP C_1 and hnRNP C_2 expression analyzed

*Corresponding authors. Haihong Shen, Tel: +82-62-715-2507; Fax: +82-62-715-2484; E-mail: haihongshen@gist.ac.kr; Xuexiu Zheng, Tel: +82-62-715-2520; Fax: +82-62-715-2484; E-mail: xuexiuzheng@gist.ac.kr
[†]Current address: New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu 41061, Korea

https://doi.org/10.5483/BMBRep.2019.52.11.080

Received 25 March 2019, Revised 3 April 2019, Accepted 3 April 2019

Keywords: Exon 11 inclusion, hnRNP C_1/C_2, Pre-mRNA splicing, Ron proto-oncogene, RRM domain

ISSN: 1976-670X (electronic edition)

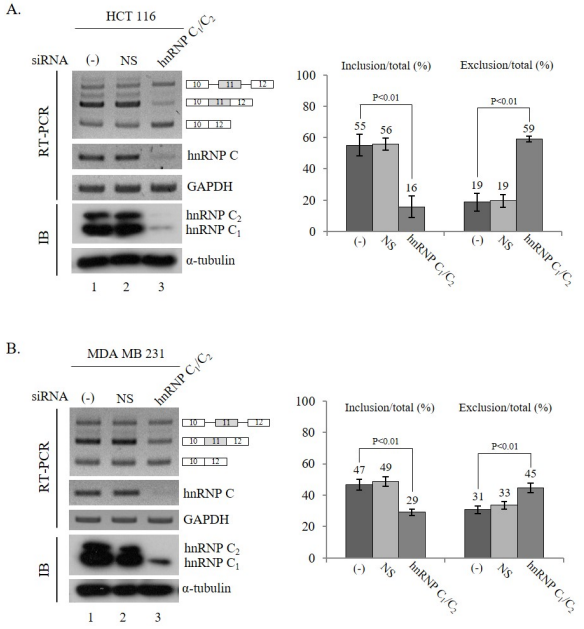

Fig. 1. Reduced hnRNP C_1/C_2 expression inhibits exon 11 splicing of Ron pre-mRNA. RT-PCR analysis of Ron exon 11 splicing was performed with RNAs extracted from untreated, non-silencing shRNA, and hnRNP C_1/C_2 shRNA lentivirus-treated HCT 116 (A) and MDA MB 231 (B) cells. The exon 11 included, skipped, and unspliced isoforms are shown as indicated. The reduced expression of hnRNP C_1/C_2 is demonstrated by RT-PCR and immunoblotting using anti-hnRNP C_1/C_2 antibody, with GAPDH and α-tubulin as controls. The quantitation results of exon 11 inclusion or exclusion in total RNA are shown in the right panel.

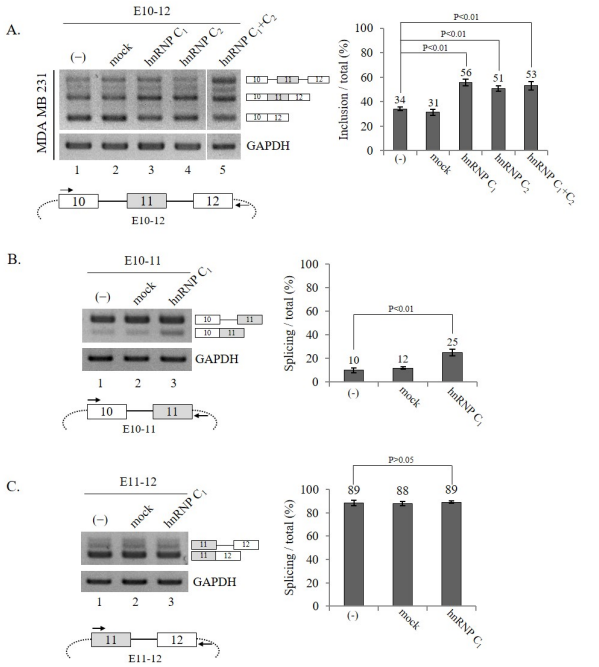

Fig. 2. hnRNP C1 and hnRNP C2 promote exon 11 splicing of Ron pre-mRNA independently. (A) (Upper panel) Shown are RT-PCR results of Ron exon 11 splicing from the E10-12 mini-gene in MDA MB 231 cells expressing hnRNP C_1, hnRNP C_2, or both hnRNP C_1 and C_2. The quantitation results are shown on the right. (Lower panel) The scheme of Ron mini-gene (E10-12) is shown. The intronic RNA is represented with solid lines and the vector sequence is shown as dot lines. The primers used to detect the mini-gene splicing are shown with arrows. (B, C) hnRNP C_1 promotes intron 10 but not intron 11 splicing of Ron pre-mRNA. (B) (Upper panel) RT-PCR analysis of intron 10 splicing from cells expressing hnRNP C_1 is shown with GAPDH as a loading control. The quantitation results are shown on the right. (Lower panel) The scheme of the E10-11 mini-gene is shown. The intronic RNA is represented with solid lines and the vector sequence is shown as dotted lines, and the primers used to detect intron 10 splicing are shown as arrows. (C) (Upper panel) RT-PCR analysis of intron 11 splicing is shown, with GAPDH as a loading control. The quantitation results are shown in the right panel. (Lower panel) The scheme of the E11-12 mini-gene is shown. The primers used to detect intron 11 splicing are shown with arrows.

by RT-PCR and immunoblotting analysis (left panel). The results in Fig. 1A show that hnRNP C_1/C_2-targeting shRNA virus treatment induced a significant decrease in the exon 11-included isoform (~39%) and an increase in the exon 11-skipped isoform (~40%) in HCT 116 cells. Thus, reduced hnRNP C_1/C_2 expression inhibited exon 11 splicing in HCT 116 cells. Similarly, as shown in Fig. 1B (left panel), shRNA-mediated knockdown also successfully reduced hnRNP C_1/C_2 expression in MDA MB 231 cells (lane 3) and reduced hnRNP C_1/C_2 expression suppressed exon 11 inclusion (~18%). Taken together, these results demonstrate that reduced expression of hnRNP C_1/C_2 inhibited exon 11 inclusion of Ron pre-mRNA.

hnRNP C_1 and C_2 promotes exon 11 of Ron pre-mRNA independently

Next, we investigated whether increased expression of hnRNP C_1 or hnRNP C_2 had the opposite effect of reduced hnRNP C_1/C_2 expression on exon 11 splicing. In order to address the question, an hnRNP C_1 or C_2 expression plasmid and Ron exon 10-12 mini-gene were co-transfected into MDA MB 231 cells. Fig. 2A shows that hnRNP C_1 and C_2 promoted an increase in exon 11 inclusion (~22% or ~17% independently, respectively) to similar levels, which was the opposite effect of hnRNP C_1/C_2 knockdown (lane 3 and 4). In addition, the results also indicate that the 13 amino acids included only in hnRNP C_2 did not play a significant role in this activity.

In addition to forming homo-tetramers by themselves, hnRNP C_1 and C_2 have also been shown to form a hetero-tetramer with a 3:1 ratio (16). We, therefore, asked if hnRNP C_1 and C_2 could promote Ron exon 11 splicing cooperatively. To answer this question, we introduced both hnRNP C_1 and C_2 expression plasmids with the Ron mini-gene

into cells. The results in Fig. 2A show that the co-expression of hnRNP C_1 and C_2 had similar effects as the individual expression of either the hnRNP C_1 or C_2 plasmid (lane 5). Thus, we conclude that these two proteins did not synergistically increase either hnRNP C_1 or C_2 expression. By combining the results in Fig. 1 and 2A, we conclude that hnRNP C_1 and C_2 functioned similarly to promote exon 11 inclusion of Ron pre-mRNA and that the 13 amino acids in hnRNP C_2 were not required for the function. To simplify our studies, we decided to examine only the roles of hnRNP C_1 in this report.

hnRNP C_1 promotes intron 10 but not intron 11 splicing of Ron pre-mRNA

We asked if hnRNP C_1 affected splicing of intron 10 or intron 11 in Ron pre-mRNA. To detect intron 10 splicing, we applied a mini-gene in which only exon 10 to exon 11 sequences were included (E10-11, lower panel, Fig. 2B). Using this mini-gene, we performed RT-PCR with primer pairs corresponding to exon 10 and the downstream vector sequence. The results in Fig. 2B demonstrate that hnRNP C_1 expression increased the intron 10-spliced isoform significantly (~15%, lane 3). Thus, hnRNP C_1 promotes intron 10 splicing. We next analyzed intron 11 splicing using another mini-gene, which includes exons 11-12 (E11-12). The primer pairs that corresponded to exon 11 and the vector sequences were used to detect intron 11 splicing (Fig. 2C, lower panel). The results in Fig. 2C show that intron 11 was almost completely spliced in the E11-12 mini-gene (lane 1). Thus, we expect that a further increase in exon 11-spliced products would be hard to detect. The results in Fig. 2C show that intron 11 splicing was not altered by the hnRNP C_1 treatment. The results in Fig. 2B and 2C indicate that hnRNP C_1 affected intron 10 but not intron 11 splicing. Therefore, we conclude that hnRNP C_1 promoted exon 11 inclusion through activation of intron 10 splicing.

Conserved splice-site sequences of exon 11 do not affect hnRNP C_1 function

We further asked if splicing sites flanking exon 11 would regulate the effect of hnRNP C_1 on the exon 11-inclusion of Ron pre-mRNA. To test this possibility, we used two previously described mutant mini-gene constructs (10) in which either the 5′ splice-site or the 3′ splice-site was mutated into conserved sequences (5′-cons or 3′-cons, Fig. 3A). Consistent with our previous reports, more conserved splice-sites sequences in exon 11 facilitated exon 11 inclusion significantly (10), leading to the predominant production of exon 11-included isoforms by these two mutants (lane 1 of Fig. 3B and 3C). However, the results in Fig. 3B demonstrate that a mutation of the 5′ splice-site in a conserved sequence did not disrupt hnRNP C_1 function on Ron exon 11 splicing because hnRNP C_1 still promoted exon 11 inclusion in the 5′-cons mutant. In the 3′-cons mutant, since the exon 11-excluded isoform was not detectable, a decrease in the exon 11-skipped form was not observable. Taken together, we conclude that conserved splice-site sequences of exon 11 did not affect the role of hnRNP C_1 in exon 11 splicing.

The Asp/Glu domain is dispensable for hnRNP C_1 function in Ron exon 11 splicing

hnRNP C_1 includes RRM, bZLM, and Asp/Glu domains. While the RRM domain is required for RNA binding, the Asp/Glu domain is required for the formation of tetramers of hnRNP C_1. To determine if these two domains were necessary for hnRNP C_1-mediated Ron exon 11 splicing, we produced two hnRNP C_1 mutants, in which either the RRM domain or the Asp/Glu domain was deleted (ΔRRM, ΔAsp/Glu) (Fig. 4A). The results in Fig. 4B show that the ΔRRM mutant protein was not able to promote exon 11 inclusion (lane 4). Thus, the RRM domain was required for the role of hnRNP C_1 in exon 11 splicing. This was not unexpected because the RRM domain is required for the binding target RNA in exon 10. In contrast, we found

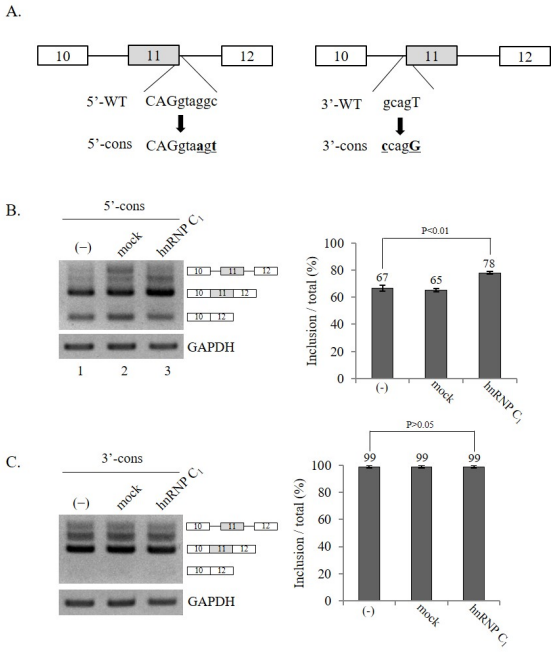

Fig. 3. Conserved splice-site sequences of exon 11 do not affect hnRNP C_1 function. (A) (Left panel) 5′ splice-site sequence of Ron exon 11 (5′-WT) and its conserved splice-site mutant sequence are shown (5′-cons). (Right panel) 3′ splice-site sequence of Ron exon 11 (3′-WT) and its conserved splice-site mutant sequence are shown (3′-cons). (B) RT-PCR analysis of exon 11 splicing using 5′-cons mini-gene in hnRNP C_1-expressing cells. GAPDH was used as a loading control. The quantitation of exon 11 inclusion in total RNA is shown. (C) RT-PCR analysis of exon 11 splicing using 3′-cons mini-gene in hnRNP C_1-expressing cells. GAPDH was used as a loading control. The quantitation of exon 11 inclusion in total RNA is shown.

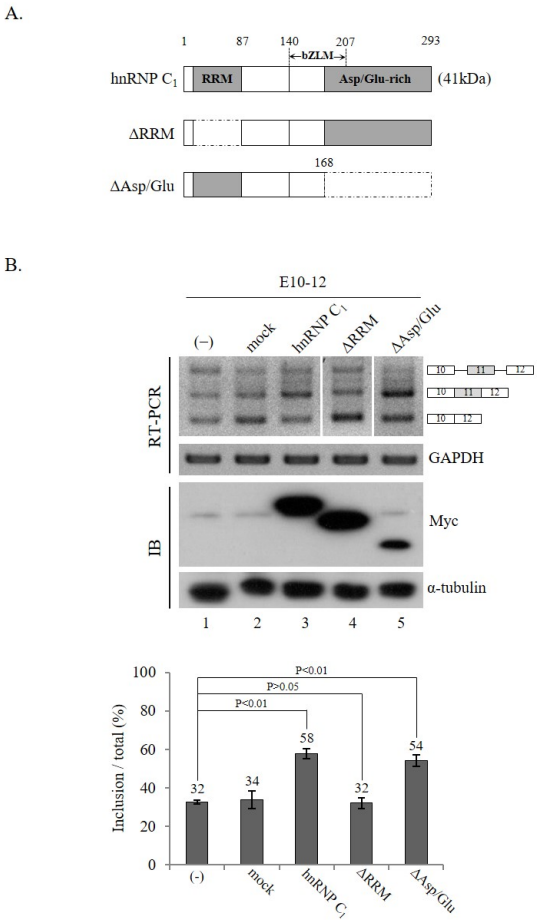

Fig. 4. The Asp/Glu domain is dispensable for the function of hnRNP C_1 in Ron exon 11 splicing. (A) Scheme of the hnRNP C_1 protein domains is shown. RRM and Asp/Glu domain are shown with gray boxes. Parts deleted in the ΔRRM and ΔAsp/Glu mutants are shown in dotted line-framed boxes. (B) RT-PCR analysis of exon 11 splicing using RNAs extracted from cells expressing ΔRRM and ΔAsp/Glu mutant proteins. Expression of myc-tagged wild-type hnRNP C_1, ΔRRM, or ΔAsp/Glu mutant protein is confirmed with anti-myc antibody. The quantitation of exon 11 inclusion in total RNA is demonstrated in the lower panel.

that the ΔAsp/Glu mutant of hnRNP C_1 was still capable of promoting exon 11 inclusion (lane 5). Therefore, we conclude that the Asp/Glu domain was not needed for the role of hnRNP C_1 in Ron exon 11 splicing. The results indicate that tetramer formation was not required for the role of hnRNP C_1 in Ron exon 11 alternative splicing.

DISCUSSION

We previously demonstrated that SRSF2 regulated Ron exon 11 splicing by contacting exon 11 sequences (10). In this study, by using shRNA-mediated knockdown and overexpression, we showed that hnRNP C_1/C_2 promoted Ron exon 11 splicing by stimulating intron 10 but not intron 11 splicing. Moreover, hnRNP C_1 and C_2 played roles in Ron exon 11 splicing independently but not synergistically, demonstrated by experiments using cells co-expressing both proteins. Furthermore, the acidic Asp/Glu domain required for tetramer formation was not necessary for the role of hnRNP C_1 in Ron splicing.

Ron pre-mRNA splicing is regulated by multiple proteins, including SRSF1, hnRNP A1, and SRSF2, through various sequences on Ron pre-mRNA (8-10). Whereas SRSF1 and hnRNP A1 target exon 12 RNA and SRSF2 targets exon 11 RNA, here, we demonstrated that hnRNP C_1 targeted exon 10 sequences. However, in spite of these studies, it is still unclear if SRSF1, hnRNP A1, SRSF2, and hnRNP C_1 function cooperatively in Ron exon 11 splicing, spliceosome assembly, and/or splice-site selection. Moreover, how and why these different proteins only target their respective sequences on Ron RNA, but not other potential binding sequences at other locations, need to be answered.

Although the role of hnRNP C_1/C_2 was demonstrated in an *in vitro* study (17), their functions in alternative splicing have not yet been elucidated in great detail. In addition, a previous study found that hnRNP C_1/C_2 was not necessary for viability (18). Thus, it is likely that hnRNP C_1/C_2 played redundant roles in splicing in the cells. In this study, we presented direct evidence that hnRNP C_1/C_2 regulated alternative splicing using cells in which hnRNP C_1/C_2 was either overexpressed or suppressed. Our results, as well as a few other reports, demonstrated that hnRNP C_1/C_2 was an essential regulatory protein of alternative splicing (19-21). However, previous studies of hnRNP C_1/C_2 were primarily based on large scale sequencing and screening, therefore, mechanistic insight from those studies was limited. The results in our study showed that reduced hnRNP C_1/C_2 expression induced a much more significant change in exon 11 splicing compared to hnRNP C_1/C_2 overexpression. The difference can be explained by the fact that hnRNP C_1/C_2 is one of the most abundant proteins in cells (11). Thus, shRNA treatment induced a significant decrease in hnRNP C_1/C_2 expression. Although we used various approaches, we were not able to show that the endogenous Ron exon 11 splicing was affected by hnRNP C_1/C_2 overexpression. Nonetheless, the transient expression of hnRNP C_1/C_2, along with the Ron mini-gene, demonstrated that it affected exon 11 splicing but to a much lesser extent than the effect seen from the knockdown.

hnRNP C_1 and C_2 are able to form homo- or heterotetramers (13). However, it seems that the role of hnRNP C_1/C_2 in Ron splicing was not required for tetramer formation, based on two pieces of evidence. First, hnRNP C_1 and C_2 did not function cooperatively in alternative splicing of Ron pre-mRNA, but rather independently. Second, the acidic Asp/Glu domain that is essential for tetramer formation was dispensable in Ron exon 11 splicing. In addition, we showed that the RRM

domain was required for the function of hnRNP C_1, which was not surprising. However, what was striking is that the long Asp/Glu domain was not necessary for Ron splicing, although it was previously shown to be essential for tetramer formation and that the hnRNP C tetramer was important for mRNA transport (22). Therefore, the role of Asp/Glu in Ron pre-mRNA splicing cannot be established. However, whether the Asp/Glu domain is required for other pre-mRNA splicing is still unknown. It is also possible that the Asp/Glu domain plays regulatory roles in alternative splicing.

MATERIALS AND METHODS

Plasmid construction

The coding region of hnRNP C_1, C_2 was inserted into a pcDNA6/myc-His A (Invitrogen) plasmid. The ΔAsp/Glu and ΔRRM mutants of hnRNP were produced by overlapping PCR using the hnRNP C_1 expression plasmid as a template.

RT-PCR

Total RNA was extracted using RiboEx (GeneAll) as previously described (23). Reverse transcription was performed using 0.5 μg RNA with oligo (dT) primer and ImProm-II™ reverse transcriptase (Promega). The reaction mixture (0.5 μl) was amplified by PCR using G-Taq polymerase (Cosmo Genetech).

Purification of hnRNP C_1 protein

Total protein was extracted from HEK293 cells transfected with the pcDNA6/myc-His A-hnRNP C_1 plasmid by 30 min incubation with lysis buffer (50 mM NaH_2PO_4, 500 mM NaCl, 5 mM imidazole, 0.5% Tween-20, and 1 mM PMSF). Prewashed Ni-NTA agarose beads (QIAGEN) were added to the lysates and the mixture was incubated overnight at 4°C in the binding buffer (50 mM NaH_2PO_4, 500 mM NaCl, 0.5% Tween-20, and 1 mM PMSF). After washing, the hnRNP C_1 protein was eluted from the Ni-NTA agarose beads using elution buffer (250 mM imidazole in binding buffer) for 20 min at 4°C.

Knockdown of hnRNP C_1/C_2 with shRNA

To generate shRNA lentivirus, 293T cells were transfected with an shRNA-harboring plasmid (Open Biosystems) and PSPAX2 and PMD2G helper plasmids using PEI reagent. The media was changed after 12 h and incubated for another 24 h. The lentivirus-containing supernatants were harvested with a 0.45 μm filter. To knock down the hnRNP C_1/C_2 expression, lentivirus-containing supernatants were added to the cells supplemented with 10 μg/ml polybrene. After 72 h infection, the RNAs were extracted for RT-PCR.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (HI17C0196).

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Wahl MC, Will CL and Luhrmann R (2009) The spliceosome: design principles of a dynamic RNP machine. Cell 136, 701-718
2. Papoff G, Cascino I, Eramo A, Starace G, Lynch DH and Ruberti G (1996) An N-terminal domain shared by Fas/Apo-1 (CD95) soluble variants prevents cell death in vitro. J Immunol 156, 4622-4630
3. Droin N, Rebe C, Bichat F, Hammann A, Bertrand R and Solary E (2001) Modulation of apoptosis by procaspase-2 short isoform: selective inhibition of chromatin condensation, apoptotic body formation and phosphatidylserine externalization. Oncogene 20, 260-269
4. Kroemer G (1997) The proto-oncogene Bcl-2 and its role in regulating apoptosis. Nat Med 3, 614-620
5. Iwama A, Okano K, Sudo T, Matsuda Y and Suda T (1994) Molecular cloning of a novel receptor tyrosine kinase gene, STK, derived from enriched hematopoietic stem cells. Blood 83, 3160-3169
6. Collesi C, Santoro MM, Gaudino G and Comoglio PM (1996) A splicing variant of the RON transcript induces constitutive tyrosine kinase activity and an invasive phenotype. Mol Cell Biol 16, 5518-5526
7. Okino T, Egami H, Ohmachi H et al (1999) Presence of RON receptor tyrosine kinase and its splicing variant in malignant and non-malignant human colonic mucosa. Int J Oncol 15, 709-714
8. Ghigna C, Giordano S, Shen H et al (2005) Cell motility is controlled by SF2/ASF through alternative splicing of the Ron protooncogene. Mol Cell 20, 881-890
9. Bonomi S, di Matteo A, Buratti E et al (2013) HnRNP A1 controls a splicing regulatory circuit promoting mesenchymal-to-epithelial transition. Nucleic Acids Res 41, 8665-8679
10. Moon H, Cho S, Loh TJ et al (2014) SRSF2 promotes splicing and transcription of exon 11 included isoform in Ron proto-oncogene. Biochim Biophys Acta 1839, 1132-1140
11. Dreyfuss G, Kim VN and Kataoka N (2002) Messenger-RNA-binding proteins and the messages they carry. Nat Rev Mol Cell Biol 3, 195-205
12. Brunner JE, Nguyen JH, Roehl HH, Ho TV, Swiderek KM and Semler BL (2005) Functional interaction of heterogeneous nuclear ribonucleoprotein C with poliovirus RNA synthesis initiation complexes. J Virol 79, 3254-3266
13. McAfee JG, Shahied-Milam L, Soltaninassab SR and LeStourgeon WM (1996) A major determinant of hnRNP C protein binding to RNA is a novel bZIP-like RNA binding domain. RNA 2, 1139-1152
14. Gorlach M, Wittekind M, Beckman RA, Mueller L and Dreyfuss G (1992) Interaction of the RNA-binding domain of the hnRNP C proteins with RNA. EMBO J 11,

3289-3295
15. Zarnack K, Konig J, Tajnik M et al (2013) Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of Alu elements. Cell 152, 453-466
16. Barnett SF, Friedman DL and LeStourgeon WM (1989) The C proteins of HeLa 40S nuclear ribonucleoprotein particles exist as anisotropic tetramers of $(C_1)3$ C_2. Mol Cell Biol 9, 492-498
17. Dreyfuss G, Matunis MJ, Pinol-Roma S and Burd CG (1993) hnRNP proteins and the biogenesis of mRNA. Annu Rev Biochem 62, 289-321
18. Williamson DJ, Banik-Maiti S, DeGregori J and Ruley HE (2000) hnRNP C is required for postimplantation mouse development but Is dispensable for cell viability. Mol Cell Biol 20, 4094-4105
19. Konig J, Zarnack K, Rot G et al (2010) iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. Nat Struct Mol Biol 17, 909-915
20. Venables JP, Koh CS, Froehlich U et al (2008) Multiple and specific mRNA processing targets for the major human hnRNP proteins. Mol Cell Biol 28, 6033-6043
21. Izquierdo JM (2010) Heterogeneous ribonucleoprotein C displays a repressor activity mediated by T-cell intracellular antigen-1-related/like protein to modulate Fas exon 6 splicing through a mechanism involving Hu antigen R. Nucleic Acids Res 38, 8001-8014
22. McCloskey A, Taniguchi I, Shinmyozu K and Ohno M (2012) hnRNP C tetramer measures RNA length to classify RNA polymerase II transcripts for export. Science 335, 1643-1646
23. Moon H, Cho S, Loh TJ et al (2017) SRSF2 directly inhibits intron splicing to suppresses cassette exon inclusion. BMB Rep 50, 423-428