Biochemical and Biophysical Research Communications 459 (2015) 29–35

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Heterogeneous nuclear ribonucleoprotein K upregulates the kinetochore complex component NUF2 and promotes the tumorigenicity of colon cancer cells

Hironobu Sugimasa [a, 1], Kenzui Taniue [a, 1], Akiko Kurimoto [a, b], Yasuko Takeda [a], Yoshihiro Kawasaki [a], Tetsu Akiyama [a, *]

[a] Laboratory of Molecular and Genetic Information, Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo, 113-0032, Japan
[b] Oncology Research Laboratories, Daiichi Sankyo Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo, 140-8710, Japan

ARTICLE INFO

Article history:
Received 3 February 2015
Available online 19 February 2015

Keywords:
Heterogeneous nuclear ribonucleoprotein K (hnRNP K)
NDC80 kinetochore complex component NUF2
Colorectal cancer
Tumorigenicity

ABSTRACT

Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a multi-functional protein involved in transcription, mRNA splicing, mRNA stabilization and translation. Although hnRNP K has been suggested to play a role in the development of many cancers, its molecular function in colorectal cancer has remained elusive. Here we show that hnRNP K plays an important role in the mitotic process in HCT116 colon cancer cells. Furthermore, we demonstrate that hnRNP K directly transactivates the NUF2 gene, the product of which is a component of the NDC80 kinetochore complex and which is known to be critical for a stable spindle microtubule-kinetochore attachment. In addition, knockdown of both hnRNP K and NUF2 caused failure in metaphase chromosome alignment and drastic decrease in the growth of colon cancer cells. These results suggest that the hnRNP K-NUF2 axis is important for the mitotic process and proliferation of colon cancer cells and that this axis could be a target for the therapy of colon cancer.

1. Introduction

Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a nucleic acid-binding protein that has three K homology (KH) domains, which recognize single-strand RNA or DNA. It has been shown that hnRNP K has multiple functions in transcription, mRNA splicing, mRNA stabilization and translation [1,2]. hnRNP K acts as both an activator and a repressor in transcriptional process. It interacts directly with the RNA polymerase machinery through its association with the TATA-box binding protein and stimulates transcription [3]. hnRNP K also interacts with factors essential for transcription, including p53, the transcriptional repressor Zik1 and androgen receptor [4–6].

hnRNP K is highly expressed in many types of cancers, including breast cancer, lung cancer, colorectal cancer, prostate cancer, pancreatic cancer, squamous cell carcinoma, nasopharyngeal carcinoma, melanoma, and chronic myeloid leukemia [2]. It is also known that hnRNP K is up-regulated by EGF signaling and that overexpression of hnRNP K increases c-Myc promoter activity in breast cancer cells [7]. In addition, it has been reported that the subcellular localization of hnRNP K is important for tumor development [8–10]. Furthermore, it has been shown that the expression levels of nuclear hnRNP K are correlated with enhanced tumor proliferation rates [11]. However, the precise molecular function of hnRNP K in tumorigenesis remains to be clarified.

The NDC80 kinetochore complex is composed of individual 4 proteins: NUF2, HEC1, SPC24 and SPC25. NUF2-HEC1 heterodimer interacts with the plus ends of spindle microtubules, whereas SPC24-SPC25 heterodimer anchors the complex into the kinetochore [12]. It has been shown that NUF2 is critical for a stable spindle microtubule-kinetochore attachment [13,14]. It has also been reported that NUF2 expression is upregulated in colon cancer, gastric cancer and lung cancer and knockdown of NUF2

Abbreviations: hnRNP K, heterogeneous nuclear ribonucleoprotein K; qRT-PCR, quantitative real-time PCR; CIN, chromosome instability.

* Corresponding author. Laboratory of Molecular and Genetic Information, Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo, 110-0032, Japan. Fax: +81 3 5841 8482.
E-mail address: akiyama@iam.u-tokyo.ac.jp (T. Akiyama).

[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.02.043
0006-291X/

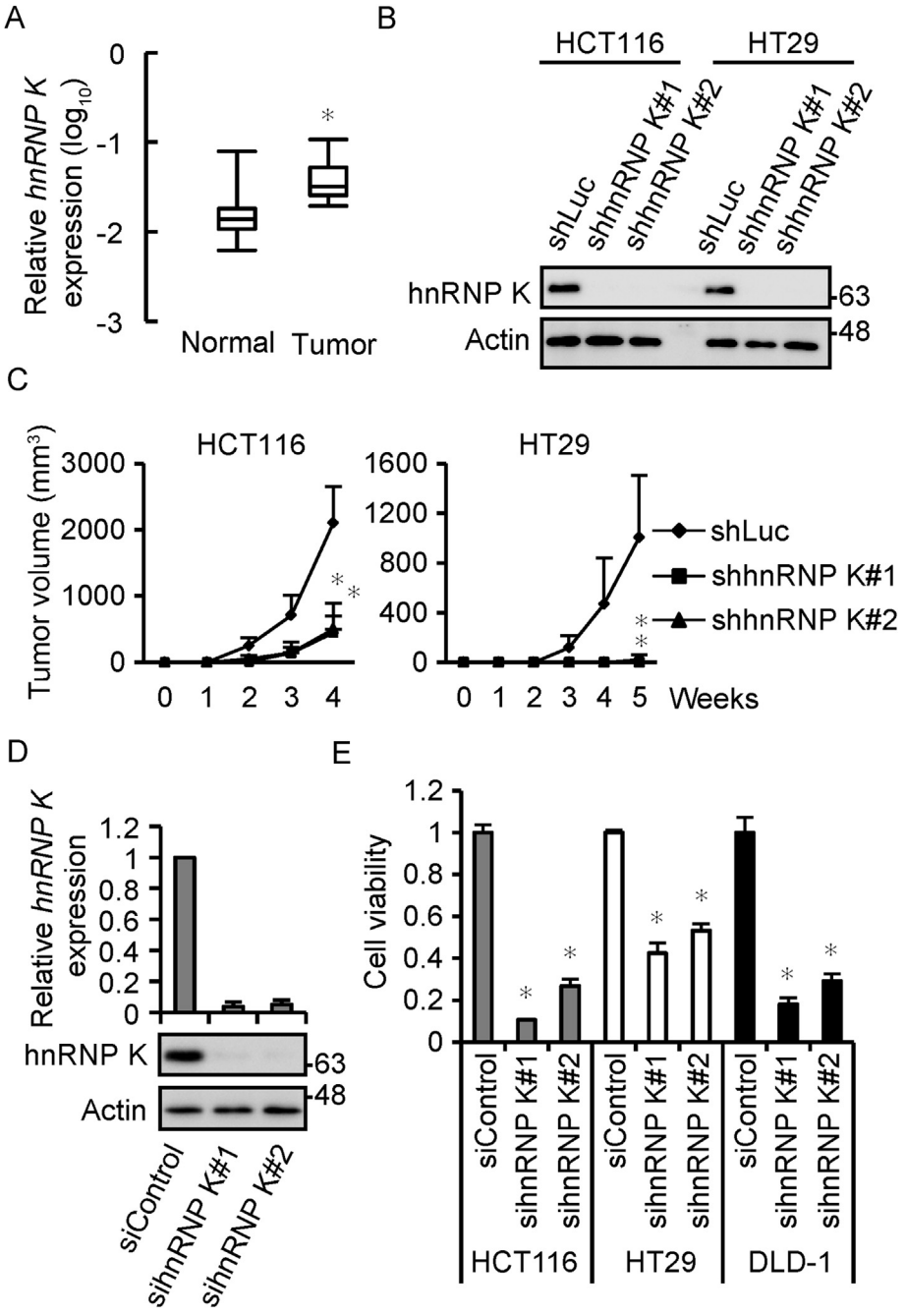

Fig. 1. hnRNP K is required for the tumorigenicity of colon cancer cell lines. (A) qRT-PCR analysis of *hnRNP K* expression in human colon cancerous and corresponding noncancerous tissues. Prior to fold-change calculation, the values were normalized to the signal generated from *β-Actin* mRNA (n = 24 pairs). $^*P < 0.05$, the Mann–Whitney *U*-test. (B) Cell lysates from HCT116 and HT29 cells infected with a lentivirus harbouring an shRNA targeting hnRNP K were subjected to immunoblotting analysis with anti-hnRNP K or anti-Actin antibody. Actin was used as a loading control. Two distinct shRNAs targeting hnRNP K were used (shhnRNP K#1, shhnRNP K#2). (C) HCT116 and HT29 cells infected with a lentivirus expressing an shRNA targeting hnRNP K were injected into nude mice. Results are expressed as the means ± s.e.m., n = 7, $^*P < 0.05$, one-sided Student's *t*-test. (D) (Upper) qRT-PCR analysis of *hnRNP K* expression in HCT116 and HT29 cells transfected with siRNA targeting hnRNP K. (Lower) Cell lysates were subjected to immunoblotting analysis with anti-hnRNP K or anti-Actin antibody. *β-Actin* mRNA was used as an internal control. Actin was used as a loading control. Results are expressed as the means ± s.e.m., n = 3. (E) Viability of HCT116, HT29 and DLD-1 cells transfected with siRNA targeting hnRNP K was assessed by Cell Titer-Glo assays. Results are expressed as the means ± s.e.m., n = 3, $^*P < 0.05$, one-sided Student's *t*-test.

expression suppresses the growth of tumor cells [15,16]. In addition, elevated NUF2 expression is associated with poor prognosis for colorectal cancer and multiple myeloma patients [16,17].

In the present study, we searched for potential down-stream targets of hnRNP K by RNA-seq analysis using the colon cancer cell line HCT116. We found that hnRNP K is involved in the mitotic process. Furthermore, we demonstrated that hnRNP K activates the transcription of the NUF2 gene and is required for the proliferation of colon cancer cell lines. These results suggest that the hnRNP K-NUF2 axis is important for the tumorigenicity of colon cancer cells.

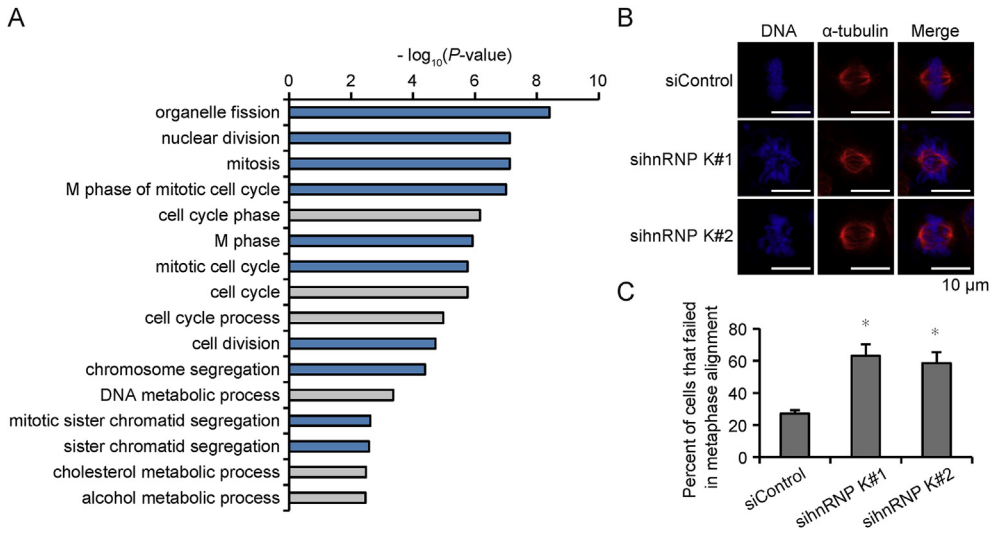

Fig. 2. hnRNP K is involved in the mitotic process. (A) GO analysis of downregulated genes in hnRNP K-depleted HCT116 cells. Blue bars indicate GO terms related to mitotic process. (B) The effect of hnRNP K knockdown on metaphase chromosome alignment in HCT116 cells was analyzed by immunostaining with anti-α-tubulin antibody (Red) and Hoechst 33258 (Blue). Scale bar, 10 μm. (C) Quantification of chromosome alignment phenotype of HCT116 cells transfected with control siRNA or siRNA targeting hnRNP K. The percentage of cells with unaligned chromosomes (5 > chromosomes off of a metaphase plate) were quantified. Results are expressed as the mean ± s.e.m., n = 3. For each condition, at least 80 cells were scored. *$P < 0.05$, one-sided Student's *t*-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Cell culture and transfection

HCT116 and HT29 cells (ATCC) were cultured in McCoy's 5-A medium supplemented with 10% fetal bovine serum. DLD-1 cells (ATCC) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Plasmids were transfected into cells using Lipofectamine LTX (invitrogen) or polyethylenimine 'MAX' (PEI, Polyscience, Inc. Cat. 24765).

2.2. Antibodies

Mouse monoclonal antibody to hnRNP K (R8903), NUF2 (sc-271251) and α-tubulin (DM1A) were obtained from Sigma, Santa Cruz and Millipore, respectively. Rabbit polyclonal antibody to actin (A2066) was obtained from Sigma. Secondary antibodies and ECL-plus were purchased from GE Healthcare.

2.3. Mice

Mouse experiments were approved by the Ethics Committee of the Institute of Molecular and Cellular Biosciences, The University of Tokyo and were performed according to 'the Guidelines for Proper Conduct of animal Experiments' provided by the Science Council of Japan. BALB/CA nude mice were obtained from CLEA Japan (Tokyo, Japan).

2.4. Tumourigenesis assays

HCT116 or HT29 cells that had been infected with a lentivirus expressing an shRNA targeting hnRNP K were injected stereotactically into 6-week-old nude mice. All animal experimental protocols were performed in accordance with the guidelines of the Animal Ethics Committee of the University of Tokyo.

2.5. Immunofluorescence analysis

72 h after siRNA transfection, HCT116 cells were fixed with 3.6% formaldehyde in PBS for 1 h at 4 °C followed by a 5 min permeabilization in PBST (PBS plus 0.2% Triton-X 100) at room temperature. To block nonspecific antibody binding, cells were incubated with 3% bovine serum albumin (BSA) in PBST for 1 h at room temperature. Primary antibody (anti-α-tubulin antibody; 1: 2,000 in PBST with 1% BSA) was incubated with the samples overnight at 4 °C, and then coverslips were washed 3 times for 5 min in PBST. Secondary antibody (Alexa fluor 594 anti-mouse IgG; 1: 500 in PBST with 1% BSA) was applied to the samples for 1 h at room temperature. Coverslips were washed 3 times for 5 min in PBST, counterstained with Hoechst 33258 at 1: 10,000 in PBS for 5 min at room temperature, and mounted in Fluoro-KEEPER Antifade Reagent (nakarai tesque). Microscopy was performed using a confocal microscope (FV1000, Olympus, Japan). Image data were obtained using Fluoview software (Olympus).

2.6. Sequence data analysis

RNA-seq samples from HCT116 cells transfected with siRNA targeting hnRNP K were sequenced using the Illumina Hiseq 2000, and raw reads were mapped to the human reference genome (hg19) using TopHat 2.0.8 (http://tophat.cbcb.umd.edu/). Gene expression levels were calculated by Cuffdiff 2 (http://cufflinks.cbcb.umd.edu/) on the alignments from TopHat 2.0.8. Genes with fewer than one fragment per kilobase of exon per million reads mapped in either hnRNP K knockdown or control samples were removed. Genes with downregulated by hnRNP K knockdown and *P*-value < 0.1 were taken as differentially expressed genes. Functional characterization of these genes was performed using DAVID [18].

2.7. Cell Titer-Glo assay

Cell viability was determined indirectly by measuring the intracellular levels of ATP using the Cell Titer-Glo Luminescent Cell

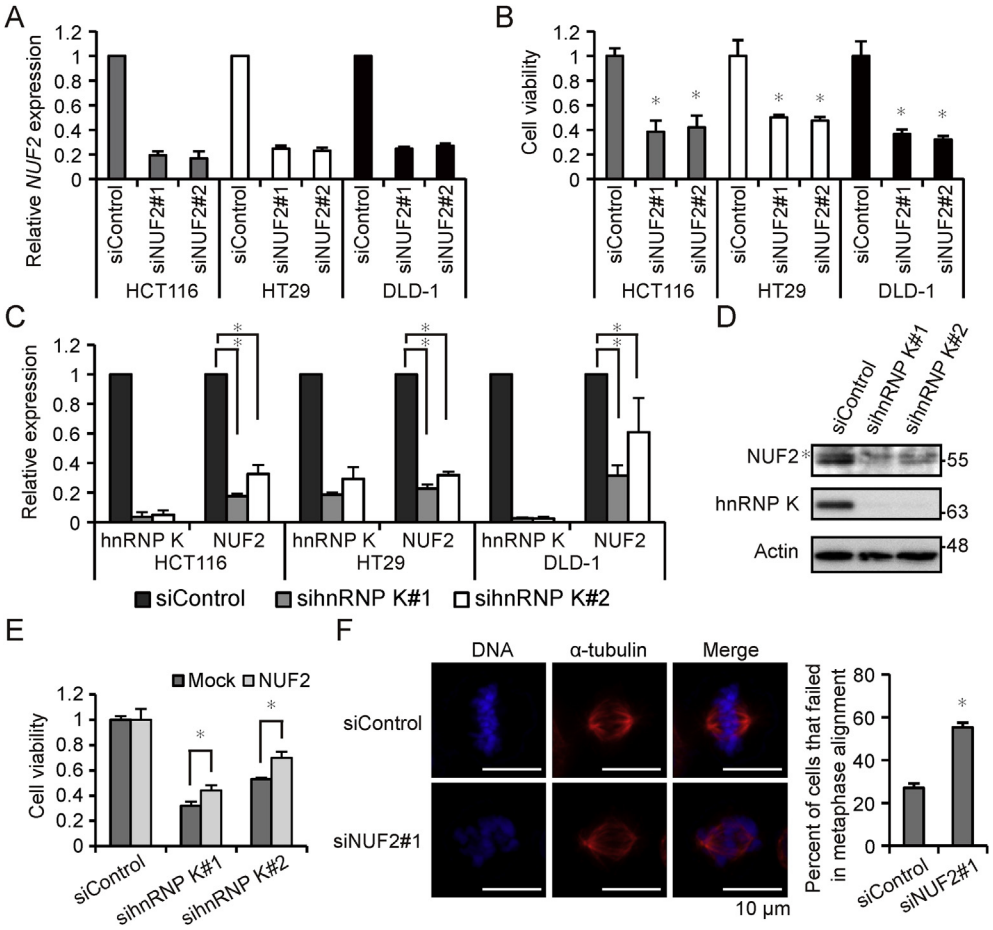

Fig. 3. NUF2 is upregulated by hnRNP K and is required for both proliferation and metaphase chromosome alignment in colon cancer cells. (A) qRT-PCR analysis of *NUF2* expression in HCT116, HT29 and DLD-1 cells transfected with siRNA targeting NUF2. *β-Actin* mRNA was used as an internal control. Results are expressed as the mean ± s.e.m., n = 3. (B) Viability of HCT116, HT29 and DLD-1 cells transfected with siRNA targeting NUF2 was assessed by Cell Titer-Glo assays. Results are expressed as the mean ± s.e.m., n = 3, $^*P < 0.05$, one-sided Student's *t*-test. (C) qRT-PCR analysis of *hnRNP K* and *NUF2* expression in HCT116, HT29 and DLD-1 cells transfected with siRNA targeting hnRNP K. *β-Actin* mRNA was used as an internal control. Results are expressed as the mean ± s.e.m., n = 3, $^*P < 0.05$, one-sided Student's *t*-test. (D) Cell lysates from HCT116 cells transfected with siRNA targeting hnRNP K were subjected to immunoblotting analysis. Actin was used as a loading control. The asterisk indicates a non-specific band. (E) Viability of HCT116 cells transfected with NUF2 or empty expression plasmid (Mock) and/or siRNA targeting hnRNP K was assessed by Cell Titer-Glo assays. Results are expressed as the mean ± s.e.m., n = 3, $^*P < 0.05$, one-sided Student's *t*-test. (F) Immunofluorescence images (Left) and quantification (Right) of HCT116 cells with unaligned chromosomes. HCT116 cells were transfected with control siRNA or siRNA targeting NUF2. Scale bar, 10 μm. Results are expressed as the mean ± s.e.m., n = 3. For each condition, at least 80 cells were scored. $^*P < 0.05$, one-sided Student's *t*-test.

Viability Assay kit (Promega). Luminescence was measured using a Mithras LB 940 (Berthold).

2.8. Luciferase assay

HCT116 cells were transfected with 50 ng of the firefly luciferase reporter and 10 ng of the pRL-tk Renilla luciferase reporter (internal control). Luciferase activities were measured using the Dual-luciferase reporter assay kit (Promega) with a luminometer (Mithras LB 940, Berthold).

2.9. Chromatin immunoprecipitation (ChIP) assay

ChIP assay was carried out essentially as described [19] using HCT116 cells. Primer sequences for quantitative PCR are listed in Supplementary Table S3.

2.10. Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test and the Student's *t*-test. The Pearson coefficient was used to measure correlations between gene expression patterns. *P*-value < 0.05 was considered to be statistically significant.

3. Results

3.1. hnRNP K is required for the tumorigenicity of colon cancer cells

It has been shown that the expression levels of hnRNP K protein are higher in colorectal cancerous tissues than in adjacent normal tissues [10]. We further investigated the mRNA levels of hnRNP K in colorectal cancer using cDNA arrays and confirmed that hnRNP K mRNA is also upregulated in colorectal cancerous tissues (Fig. 1A). To assess the significance of hnRNP K in colorectal tumourigenesis, we knocked down hnRNP K expression in HCT116 or HT29 colon cancer cells by infection with a lentivirus expressing a small hairpin RNA (shRNA) targeting hnRNP K (Fig. 1B, Supplementary Fig. S1). When stably expressing cells were transplanted into nude mice, tumor growth was significantly retarded compared with cells infected with a control lentivirus (Fig. 1C). In addition, CellTiter-Glo assays revealed that knockdown of hnRNP K by siRNA caused a significant reduction in the growth of HCT116, HT29 and DLD-1

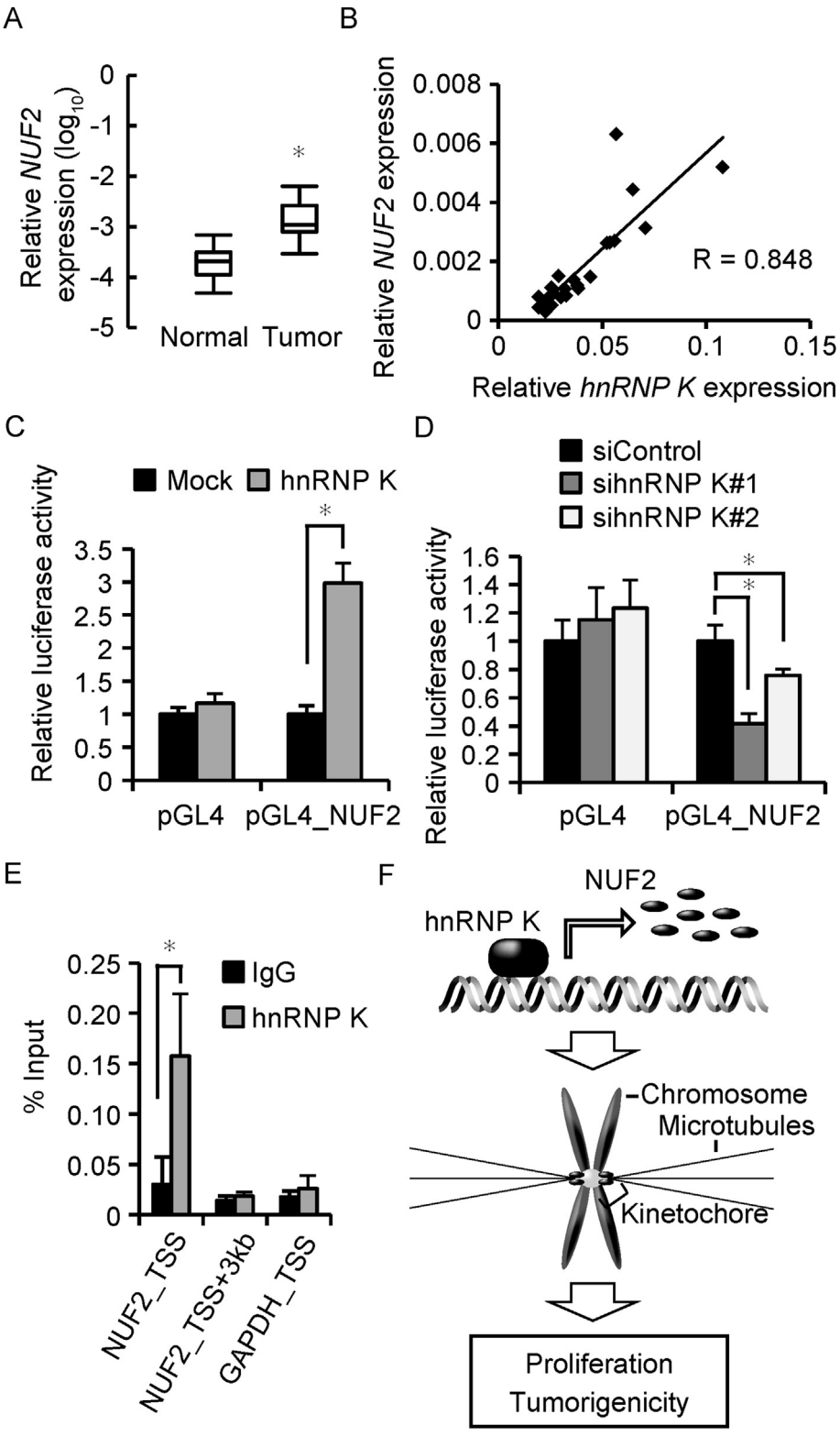

Fig. 4. hnRNP K binds to the promoter region of NUF2 and activates its transcription. (A) qRT-PCR analysis of *NUF2* expression in human colon cancerous and corresponding noncancerous tissues. Prior to fold-change calculation, the values were normalized to the signal generated from *β-Actin* mRNA (n = 24 pairs). $^{*}P < 0.05$, the Mann–Whitney *U*-test. (B) Pearson correlation scatter plot of *hnRNP K* and *NUF2* expression in human colon cancerous tissues (n = 24) (R = 0.8479, *P*-value = 1.69E-7). The values were normalized to the signal generated from *β-Actin* mRNA. (C) Luciferase assays were performed with HCT116 cells transfected with a reporter plasmid containing the NUF2 promoter region (+480 ~ −460), and the Flag-tagged hnRNP K or the empty expression plasmid (Mock). Bars represent luciferase activities in cells transfected with Flag-tagged hnRNP K divided by those in cells transfected with the empty plasmid. The pRL-tk Renilla luciferase reporter was cotransfected to normalize transfection efficiency. Results are expressed as the mean ± s.e.m., n = 3, $^{*}P < 0.05$, one-sided Student's *t*-test. (D) Luciferase assays were performed with HCT116 cells transfected with a reporter plasmid containing the NUF2 promoter region and/or siRNA targeting hnRNP K. Results are expressed as the mean ± s.e.m., n = 3, $^{*}P < 0.05$, one-sided Student's *t*-test. (E) ChIP assays were performed on HCT116 cells using an anti-hnRNP K antibody or anti-rabbit IgG. The regions around +3000 from the NUF2 transcription start site (TSS) and GAPDH TSS were amplified as negative controls. Results are expressed as the mean ± s.e.m., n = 3, $^{*}P < 0.05$, one-sided Student's *t*-test. (F) Schematic illustration of the hnRNP K-NUF2 axis in colorectal cancer.

colon cancer cells (Fig. 1D and E). These results suggest that hnRNP K is required for the proliferation and tumourigenicity of colon cancer cells.

3.2. hnRNP K is involved in the mitotic process

To clarify the molecular function of hnRNP K in colon cancer cells, we compared gene expression profiles between HCT116 cells and those in which hnRNP K expression had been suppressed by siRNA. RNA-seq analysis and consequent pathway analysis indicated that hnRNP K may be involved in the mitotic process (Fig. 2A, Supplementary Tables S1 and S2). Indeed, immunofluorescence analysis revealed that HCT116 cells that had been transfected with an siRNA targeting hnRNP K failed to align their chromosomes at the spindle equator at metaphase (Fig. 2B). Nearly 60% of the hnRNP K-depleted cells exhibited unaligned chromosomes (defined as having > 5 chromosomes off a metaphase plate), compared with only approximately 25% of cells transfected with control siRNA did (Fig. 2C). This result suggests that hnRNP K is required for metaphase chromosome alignment and proper mitotic progression in HCT116 cells.

3.3. hnRNP K upregulates the NDC80 kinetochore complex component NUF2

To identify potential target genes of hnRNP K involved in the growth of cancer cells, we performed an siRNA screen against genes that were observed to be upregulated by hnRNP K and that were related to mitotic process. Among 10 such candidate genes, knockdown of the NDC80 kinetochore complex component NUF2 by siRNA was found to cause the most significant decrease in the growth of HCT116, HT29 and DLD-1 cells (Fig. 3A and B, Supplementary Fig. S2A). We also found that knockdown of hnRNP K by siRNA resulted in decreased expression of NUF2 mRNA and protein in these tumor cells (Fig. 3C and D). Furthermore, transient expression of NUF2 partially restored the growth of HCT116 cells that had been transfected with siRNA targeting hnRNP K (Fig. 3E).

To clarify the phenotype of HCT116 cells transfected with an siRNA targeting NUF2, we performed immunofluorescence analysis. As well as hnRNP K, NUF2-depleted HCT116 cells failed to align chromosomes at metaphase (Fig. 3F, Supplementary Fig. S2B). These results suggest that hnRNP K regulates metaphase chromosome alignment and proliferation by upregulating NUF2 expression in colon cancer cells.

3.4. NUF2 expression levels are strongly correlated with those of hnRNP K

Consistent with previous studies [16], qRT-PCR analysis showed that *NUF2* mRNA was upregulated in colon cancerous tissue compared with adjacent normal tissues (Fig. 4A). Furthermore, *NUF2* expression was strongly correlated with *hnRNP K* expression (Fig. 4B). These results are consistent with our notion that the hnRNP K-NUF2 axis is important for the development of colorectal cancer.

3.5. hnRNP K binds to the promoter region of NUF2 and activates its transcription

To clarify the mechanisms underlying hnRNP K-mediated NUF2 upregulation, we performed reporter assays using a construct in which a fragment of the NUF2 promoter region was inserted upstream of the luciferase gene (pGL4-NUF2). When reporters were transfected into HCT116 cells, the activities of the pGL4-NUF2 reporter, but not the pGL4 reporter (pGL4), were enhanced by co-expression of hnRNP K (Fig. 4C). Conversely, knockdown of hnRNP K reduced NUF2 promoter activity (Fig. 4D). Furthermore, chromatin immunoprecipitation assays on HCT116 cells using anti-hnRNP K antibody revealed that hnRNP K was associated with the NUF2 promoter region (NUF2_TSS) (Fig. 4E). The region around +3000 downstream from the transcription start site (TSS) of the NUF2 gene (NUF2_TSS+3 kb) and the TSS of the GAPDH gene (GAPDH_TSS) were not immunoprecipitated in the same experiment. Taken together, these results suggest that hnRNP K directly upregulates *NUF2* expression at the transcriptional level by binding to the NUF2 promoter region.

4. Discussion

In the present study, we have shown that hnRNP K is required for the tumorigenicity of colon cancer cells. Furthermore, our results showed that hnRNP K plays an essential role in metaphase chromosome alignment and proliferation by transactivating NUF2 in HCT116 cells (Fig. 4F). On the other hand, we failed to observe upregulation of known target genes such as c-Myc and eIF4E (Supplementary Fig. S3) [7,20,21]. Thus, hnRNP K may have different functions depending on the type of tumor. In addition, this is the first report of a transcriptional factor that regulates NUF2 expression, which is known to be upregulated in several cancers and associated with the proliferation of tumor cells and poor prognosis for colorectal cancer patients [15,16].

Genomic instability is a characteristic of most human cancers and is believed to drive tumor progression [22,23]. We found that the hnRNP K-NUF2 axis may be involved in chromosome instability (CIN), wherein an increased rate of chromosome mis-segregation in mitosis results in an abnormal chromosome number (aneuploidy). Several studies have shown that CIN has both tumor-promoting and tumor-suppressive effects [24]. Low rates of CIN drive tumor progression by accelerating the gain of oncogenic and the loss of tumor suppressor loci, while high and excessive rates of CIN results in cell death or senescence. Indeed, 25% of HCT116 cells that had been transfected with control siRNA exhibited unaligned chromosomes. Thus, it is likely that the increased rates of CIN caused by knockdown of hnRNP K or NUF2 was responsible for the observed reduction in the growth of colon cancer cells.

Overexpression of NUF2 only partially restored the growth of HCT116 cells in which hnRNP K had been knocked down. This result suggests that genes other than NUF2 are also important for the growth of HCT116 cells. Interestingly, our RNA-seq analysis revealed that hnRNP K also upregulates several mitosis-related genes such as SMC1A and SGOL1. These genes are known to regulate DNA replication and mitotic process [25,26]. Moreover, it has recently been reported that various mutant SMC1As drive CIN in adenomas [27]. It has also been shown that down-regulation of SGOL1 increases CIN and promotes tumor formation [28]. It is therefore possible that not only NUF2 but also these genes may play important roles in mitotic process and proliferation of colon cancer cells.

In conclusion, we found that hnRNP K upregulates NUF2 expression and that the hnRNP K-NUF2 axis is important for the proliferation of colon cancer cells. We speculate that this axis could be a promising target for the therapy of colorectal cancer.

Conflict of interest

The authors have declared no conflicts of interest.

Acknowledgments

This work was supported by Research Program of Innovative Cell Biology by Innovative Technology (Integrated Systems Analysis of Cellular Oncogenic Signaling Networks), Grants-in-Aid for Scientific Research on Innovative Areas (Integrative Research on Cancer Microenvironment Network), Project for Development of Innovative Research on Cancer Therapeutics, Takeda Science Foundation and in part by Global COE Program (Integrative Life Science Based on the Study of Biosignaling Mechanisms), MEXT, Japan.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.043.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.043.

References

[1] K. Bomsztyk, O. Denisenko, J. Ostrowski, hnRNP K: one protein multiple processes, Bioessays 26 (2004) 629–638.
[2] P. Barboro, N. Ferrari, C. Balbi, Emerging roles of heterogeneous nuclear ribonucleoprotein K (hnRNP K) in cancer progression, Cancer Lett. 352 (2014) 152–159.
[3] E.F. Michelotti, G.A. Michelotti, A.I. Aronsohn, D. Levens, Heterogeneous nuclear ribonucleoprotein K is a transcription factor, Mol. Cell. Biol. 16 (1996) 2350–2360.
[4] A. Moumen, P. Masterson, M.J. O'Connor, S.P. Jackson, hnRNP K: an HDM2 target and transcriptional coactivator of p53 in response to DNA damage, Cell 123 (2005) 1065–1078.
[5] O.N. Denisenko, B. O'Neill, J. Ostrowski, I. Van Seuningen, K. Bomsztyk, Zik1, a transcriptional repressor that interacts with the heterogeneous nuclear ribonucleoprotein particle K protein, J. Biol. Chem. 271 (1996) 27701–27706.
[6] P. Barboro, E. Repaci, N. Ferrari, A. Rubagotti, F. Boccardo, C. Balbi, Androgen receptor and heterogeneous nuclear ribonucleoprotein K colocalize in the nucleoplasm and are modulated by bicalutamide and 4-hydroxy-tamoxifen in prostatic cancer cell lines, Prostate 71 (2011) 1466–1479.
[7] M. Mandal, R. Vadlamudi, D. Nguyen, R.A. Wang, L. Costa, R. Bagheri-Yarmand, J. Mendelsohn, R. Kumar, Growth factors regulate heterogeneous nuclear ribonucleoprotein K expression and function, J. Biol. Chem. 276 (2001) 9699–9704.
[8] Y. Guo, J. Zhao, J. Bi, Q. Wu, X. Wang, Q. Lai, Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a tissue biomarker for detection of early hepatocellular carcinoma in patients with cirrhosis, J. Hematol. Oncol. 5 (2012) 37.
[9] P. Barboro, E. Repaci, A. Rubagotti, S. Salvi, S. Boccardo, B. Spina, M. Truini, C. Introini, P. Puppo, N. Ferrari, G. Carmignani, F. Boccardo, C. Balbi, Heterogeneous nuclear ribonucleoprotein K: altered pattern of expression associated with diagnosis and prognosis of prostate cancer, Br. J. Cancer 100 (2009) 1608–1616.
[10] F. Wang, P. Zhang, C. Shi, Y. Yang, H. Qin, Immunohistochemical detection of HSP27 and hnRNP K as prognostic and predictive biomarkers for colorectal cancer, Med. Oncol. 29 (2012) 1780–1788.
[11] J. Ostrowski, K. Bomsztyk, Nuclear shift of hnRNP K protein in neoplasms and other states of enhanced cell proliferation, Br. J. Cancer 89 (2003) 1493–1501.
[12] X. Wan, R.P. O'Quinn, H.L. Pierce, A.P. Joglekar, W.E. Gall, J.G. DeLuca, C.W. Carroll, S.T. Liu, T.J. Yen, B.F. McEwen, P.T. Stukenberg, A. Desai, E.D. Salmon, Protein architecture of the human kinetochore microtubule attachment site, Cell 137 (2009) 672–684.
[13] L.J. Sundin, G.J. Guimaraes, J.G. Deluca, The NDC80 complex proteins Nuf2 and Hec1 make distinct contributions to kinetochore-microtubule attachment in mitosis, Mol. Biol. Cell. 22 (2011) 759–768.
[14] D. Liu, X. Ding, J. Du, X. Cai, Y. Huang, T. Ward, A. Shaw, Y. Yang, R. Hu, C. Jin, X. Yao, Human NUF2 interacts with centromere-associated protein E and is essential for a stable spindle microtubule-kinetochore attachment, J. Biol. Chem. 282 (2007) 21415–21424.
[15] S. Hayama, Y. Daigo, T. Kato, N. Ishikawa, T. Yamabuki, M. Miyamoto, T. Ito, E. Tsuchiya, S. Kondo, Y. Nakamura, Activation of CDCA1-KNTC2, members of centromere protein complex, involved in pulmonary carcinogenesis, Cancer Res. 66 (2006) 10339–10348.
[16] Y. Kobayashi, A. Takano, Y. Miyagi, E. Tsuchiya, H. Sonoda, T. Shimizu, H. Okabe, T. Tani, Y. Fujiyama, Y. Daigo, Cell division cycle-associated protein 1 overexpression is essential for the malignant potential of colorectal cancers, Int. J. Oncol. 44 (2014) 69–77.
[17] M. van Duin, A. Broyl, Y. de Knegt, H. Goldschmidt, P.G. Richardson, W.C. Hop, B. van der Holt, D. Joseph-Pietras, G. Mulligan, R. Neuwirth, S.S. Sahota, P. Sonneveld, Cancer testis antigens in newly diagnosed and relapse multiple myeloma: prognostic markers and potential targets for immunotherapy, Haematologica 96 (2011) 1662–1669.
[18] D.W. Huang, B.T. Sherman, Q. Tan, J. Kir, D. Liu, D. Bryant, Y. Guo, R. Stephens, M.W. Baseler, H.C. Lane, R.A. Lempicki, DAVID bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists, Nucleic Acids Res. 35 (2007) W169–W175.
[19] K. Matsuura, T. Jigami, K. Taniue, Y. Morishita, S. Adachi, T. Senda, A. Nonaka, H. Aburatani, T. Nakamura, T. Akiyama, Identification of a link between Wnt/beta-catenin signalling and the cell fusion pathway, Nat. Commun. 2 (2011) 548.
[20] G.A. Michelotti, E.F. Michelotti, A. Pullner, R.C. Duncan, D. Eick, D. Levens, Multiple single-stranded cis elements are associated with activated chromatin of the human c-myc gene in vivo, Mol. Cell. Biol. 16 (1996) 2656–2669.
[21] M. Lynch, L. Chen, M.J. Ravitz, S. Mehtani, K. Korenblat, M.J. Pazin, E.V. Schmidt, hnRNP K binds a core polypyrimidine element in the eukaryotic translation initiation factor 4E (eIF4E) promoter, and its regulation of eIF4E contributes to neoplastic transformation, Mol. Cell. Biol. 25 (2005) 6436–6453.
[22] S. Negrini, V.G. Gorgoulis, T.D. Halazonetis, Genomic instability–an evolving hallmark of cancer, Nat. Rev. Mol. Cell. Biol. 11 (2010) 220–228.
[23] C. Lengauer, K.W. Kinzler, B. Vogelstein, Genetic instabilities in human cancers, Nature 396 (1998) 643–649.
[24] B. Orr, D.A. Compton, A double-edged sword: how oncogenes and tumor suppressor genes can contribute to chromosomal instability, Front. Oncol. 3 (2013) 164.
[25] Y. Watanabe, P. Nurse, Cohesin Rec8 is required for reductional chromosome segregation at meiosis, Nature 400 (1999) 461–464.
[26] T.S. Kitajima, S.A. Kawashima, Y. Watanabe, The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis, Nature 427 (2004) 510–517.
[27] F. Cucco, A. Servadio, V. Gatti, P. Bianchi, L. Mannini, A. Prodosmo, E. De Vitis, G. Basso, A. Friuli, L. Laghi, S. Soddu, G. Fontanini, A. Musio, Mutant cohesin drives chromosomal instability in early colorectal adenomas, Hum. Mol. Genet. 23 (2014) 6773–6778.
[28] M. Iwaizumi, K. Shinmura, H. Mori, H. Yamada, M. Suzuki, Y. Kitayama, H. Igarashi, T. Nakamura, H. Suzuki, Y. Watanabe, A. Hishida, M. Ikuma, H. Sugimura, Human Sgo1 downregulation leads to chromosomal instability in colorectal cancer, Gut 58 (2009) 249–260.