European Review for Medical and Pharmacological Sciences 2023; 27: 2561-2570

METTL3-mediated downregulation of splicing factor SRSF11 is associated with carcinogenesis and poor survival of cancer patients

J. OH[1], J.-M. OH[2,3], S.-Y. CHO[1,4,5,6]

[1]Department of Translational Medicine, Seoul National University College of Medicine, Seoul, South Korea
[2]Department of Oral Biochemistry, Dental and Life Science Institute, School of Dentistry, Pusan National University, Yangsan, South Korea
[3]Department of Life Science in Dentistry, School of Dentistry, Pusan National University, Yangsan, South Korea
[4]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, South Korea
[5]Medical Research Center, Genomic Medicine Institute, Seoul National University College of Medicine, Seoul, South Korea
[6]Cancer Research Institute, Seoul National University, Seoul, South Korea

Abstract. – OBJECTIVE: N^6-methyladenosine (m^{6}A) is one of the most abundant post-transcriptional modifications in eukaryotic RNA. As m^{6}A modifications play an important role in RNA processing, abnormal m^{6}A regulation caused by aberrant expression of m^{6}A regulators is closely related to carcinogenesis. In this study, we aimed to determine the role of METTL3 expression in carcinogenesis, regulation of splicing factor expression by METTL3, and their effects in survival period and cancer-related metabolisms.

MATERIALS AND METHODS: We investigated the correlation between each splicing factor and METTL3 in breast invasive ductal carcinoma (BRCA), colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD) and gastric adenocarcinoma (STAD). Survival analysis was performed based on the expression of each splicing factor. To determine the molecular mechanism of SRSF11 in carcinogenesis, gene set enrichment analysis using RNA sequencing data was performed according to SRSF11 expression.

RESULTS: Among the 64 splicing factors used for correlation analysis, 13 splicing factors showed a positive correlation with METTL3 in all four cancer types. We found that when METTL3 expression was decreased, the expression of SRSF11 was also decreased in all four types of cancer tissue when compared to that in normal tissue. Decreased SRSF11 expression was associated with poor survival in patients with BRCA, COAD, LUAD, and STAD. Gene set enrichment analysis according to SRSF11 expression showed that the p53/apoptosis, inflammation/immune response, and ultraviolet/reactive oxygen species stimulus-response pathways were enriched in cancers with decreased SRSF11 expression.

CONCLUSIONS: These results suggest that METTL3 regulates SRSF11 expression, which could influence mRNA splicing in m^{6}A modified cancer cells. METTL3-mediated downregulation of SRSF11 expression in cancer patients correlates with poor prognosis.

Key Words:
Methyltransferase-like 3, RNA splicing, Carcinogenesis, Splicing factor, SRSF11.

Introduction

Among several epigenetic modifications, N^6-methyladenosine (m^{6}A) is the most common post-transcriptional modification in eukaryotic RNA. m^{6}A RNA modification affects RNA metabolism, such as degradation, splicing, translation, and transport[1], which are finely controlled by several complexes, such as m^{6}A writer, reader, and eraser complexes. Therefore, abnormal m^{6}A modification caused by the mutation or aberrant expression of these complexes is closely related to carcinogenesis in several cancer types[2]. m^{6}A modification is carried out by a methyltransferase writer complex comprising methyltransferase-like (METTL)3, METTL14,

Corresponding Authors: Jung-Min Oh, Ph.D; e-mail: jminoh@pusan.ac.kr;
Sung-Yup Cho, MD; e-mail: csybio@snu.ac.kr

Wilms tumor 1 associated protein (WTAP), KIAA1429, and several appendage genes[3-5]. The m^{6}A modification readers (YTHDC1, HNRNPA2B1, YTHDF1, YTHDF2, YTHDF3, and YTHDC2) recognize and bind to m^{6}A. Then, they regulate mRNA splicing, localization, translation, and decay[6-11]. Finally, m^{6}A modification is removed by demethylases/erasers such as FTO and ALKBH5[12,13].

Pre-mRNA splicing to produce mature mRNA is one of the most essential steps in eukaryotic RNA processing. In alternative splicing, various mRNAs are produced from one pre-mRNA, which causes an increase in protein diversity without increasing total genome size. Splicing is finely controlled by many factors. Therefore, if it is not properly managed, it can cause severe diseases such as cancer[14-16]. Splicing is regulated by trans-acting splicing factors that specifically bind to cis-acting elements in pre-mRNA and can also be altered by RNA mutation and modification, including m^{6}A RNA modification. Therefore, the change in m^{6}A writer, reader, and eraser complex expressions affect not only RNA expression, but also alternative splicing. Decreased METTL3, ALKBH5, or FTO expression affects alternative splicing of thousands of genes[5,13,17,18]. It is also known that YTHDC1 (m^{6}A reader) regulates mRNA splicing by modulating SRSF3 and SRSF10 splicing factors[19]. However, it is not clearly understood whether METTL3 methyltransferase affects the expression of other splicing factors. To determine the relationship between METTL3 and each splicing factor in cancer development, we analyzed the RNA expression profiles in breast invasive ductal carcinoma (BRCA), colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), and gastric adenocarcinoma (STAD).

Materials and Methods

Correlation Between METTL3 and Various Splicing Factors

Messenger RNA expression data of normal and cancer tissues of the breast, colon, lung, and stomach were obtained from the TCGA TARGET GTEx cohort in UCSC Xena (https://xenabrowser.net/datapages/). The list of splicing factors was downloaded from the SpliceAid-F database (http://www.caspur.it/SpliceAidF/)[20]. Pearson correlation coefficients between METTL3 and each splicing factor were determined using $\log_2$ (FPKM-UQ + 1) of mRNA expression levels extracted from the GDC TCGA cohorts of BRCA, COAD, LUAD, and STAD (https://xenabrowser.net/datapages/). Using R (version 4.1.0, http://www.R-project.org) and the "ggplot2" package, each coefficient was plotted as a dot on graphs as relevant. A Venn diagram ("ggvenn" package) was drawn to represent the common relationship between METTL3 and each splicing factor in the four adenocarcinomas.

Analysis of Differentially Expressed Genes Between Cancer and Normal Tissues

Differential gene expression was analyzed as previously described[21]. To compare METTL3 and splicing factor expression of normal tissues with that of cancer tissues, we used $\log_2$ (FPKM + 0.001) from the cohort. To compensate for the small number of normal tissue samples in the cohort, we used the Wilcoxon rank-sum test for testing the null hypothesis. We calculated fold change (FC) to compare the gene expression of tumor tissues with that of normal tissues as follows:

$$FC = \frac{\text{Mean expression}_{\text{tumor tissues}}}{\text{Mean expression}_{\text{normaltissues}}}$$

Scatter plots of FC and *p*-value data were generated using "ggplot2" package in R.

Survival Analysis According to Gene Expression

Survival data were analyzed as follow[21]: the survival information of all patients was extracted from the TCGA cohort (TCGA breast cancer, TCGA colon cancer, TCGA lung adenocarcinoma, and TCGA stomach cancer at https://xenabrowser.net/datapages/). As average level and distribution of mRNA expression by genes are very complex in cancer, we used maximally selected rank statistics with several *p*-value approximations to optimize the cut-off value, which was obtained from the 'maxstat' package in R (https://cran.r-project.org/web/packages/maxstat/index.html).

Survival based on gene expression was analyzed using the Kaplan–Meier method and two-sided log-rank test. Kaplan–Meier plots were plotted using "survival" (https://cran.r-project.org/web/packages/survival/index.html) and "survminer" package in R (https://cran.r-project.org/web/packages/survminer/index.html).

Gene Set Enrichment Analysis (GSEA)

To elucidate the underlying molecular mechanism of prognosis by specific splicing factors, GSEA was performed on patients with high and low splicing factor expressions. The optimized cutoff value obtained by survival analysis was used to differentiate patients with low and high expressions. We downloaded the GSEA software from the GSEA website (https://www.gsea-msigdb.org/gsea/index.jsp). Hallmark gene sets and KEGG pathway gene sets were used for the analysis. Normalized enrichment scores and false discovery rate-adjusted *q*-values obtained by GSEA were used to interpret the pathway enrichment.

Statistical Analysis

Statistical calculations were performed using R (version 4.1.0, http://www.R-project.org). Differences of gene expression between normal and cancer tissues were assessed by Wilcoxon rank-sum test. The difference was considered significant if the *p*-value was lower than 0.05.

Results

Correlation Between METTL3 and Each Splicing Factor in the Four Types of Cancer

We hypothesized that in addition to the direct splicing effect on m^{6}A modified RNAs by reader proteins, increased alternative splicing in cancer cells with altered m^{6}A modification is associated with changes in splicing factors *via* METTL3 regulation in carcinogenesis. To explore the relationship between METTL3 and each splicing factor in the four types of cancer, we analyzed the expression of METTL3 and various splicing factors in BRCA, COAD, LUAD, and STAD. A total of 64 splicing factors downloaded from the SpliceAid-F database[20] were used to compare their correlation with METTL3 (**Supplementary Table I**). The graph in Figure 1 represents the correlation between METTL3 and each splicing factor in the four cancer types. The top five splicing factors that had strong positive or negative correlations with METTL3 in each cancer type are listed in the graph (Figure 1). The total number of splicing factors with a positive correlation was higher than those with a negative correlation with METTL3. Among the splicing factors used for the analysis, 4-30 of them showed statistically significant positive or negative correlations with METTL3 (**Supplementary Figure 1**). By drawing Venn diagram, we interpreted those 13 and 1 splicing factors had positive and negative correlations with METTL3 expression, respectively (**Supplementary Figure 1**). We focused on the 13 splicing factors that were positively correlated with METTL3 in all the four types of cancer and further analyzed their role in carcinogenesis.

Decreased METTL3 Expression in Four Cancer Types

To investigate the expression levels of METTL3 and the 13 splicing factors in normal and primary tumors, we analyzed the transcriptome data of normal and cancerous tissues from four organ types (breast, colon, lung, and stomach). METTL3 expression was substantially decreased in all four cancer tissues when compared to that in normal tissues (Figure 2A). We also analyzed the expression patterns of the 13 splicing factors in the four normal and cancer tissues (Figure 2B). Interestingly, the expression of 13 splicing factors with decreased *METTL3* expression showed a tendency to be downregulated in cancer tissues when compared to that in normal tissues. Among the 13 splicing factors, *SRSF11*, *SRSF5*, and *RBM5* expression were substantially decreased in all four cancer types (Figure 2B).

Decreased SRSF11 Expression Predicts Poor Survival in Cancer

To monitor whether the 13 splicing factors influence patient survival, we analyzed the survival data of cancer patients according to the 13 splicing factor expressions in each cancer type (Figure 3A). The effect of each splicing factor on patient survival varied depending on cancer type (Figure 3A). However, decreased expression of some splicing factors showed a consistent poor survival trend in specific cancer types. Interestingly, decreased SRSF11 expression resulted in poor survival of patients with any of the four cancer types (Figures 3A and B). In addition to decreased SRSF11 expression, decreased TRA2A expression was positively correlated with poor survival of patients with BRCA, LUAD, or STAD (Figure 3A and **Supplementary Figure 2**). Decreased SRSF6 and SRSF5 expression resulted in poor survival of patients with 2 types of cancer (Figure 3A and **Supplementary Figure 2**).

GSEA According to SRSF11 Expression

Based on the expression correlation and survival analysis, SRSF11 was found to be the splicing factor substantially associated with decreased

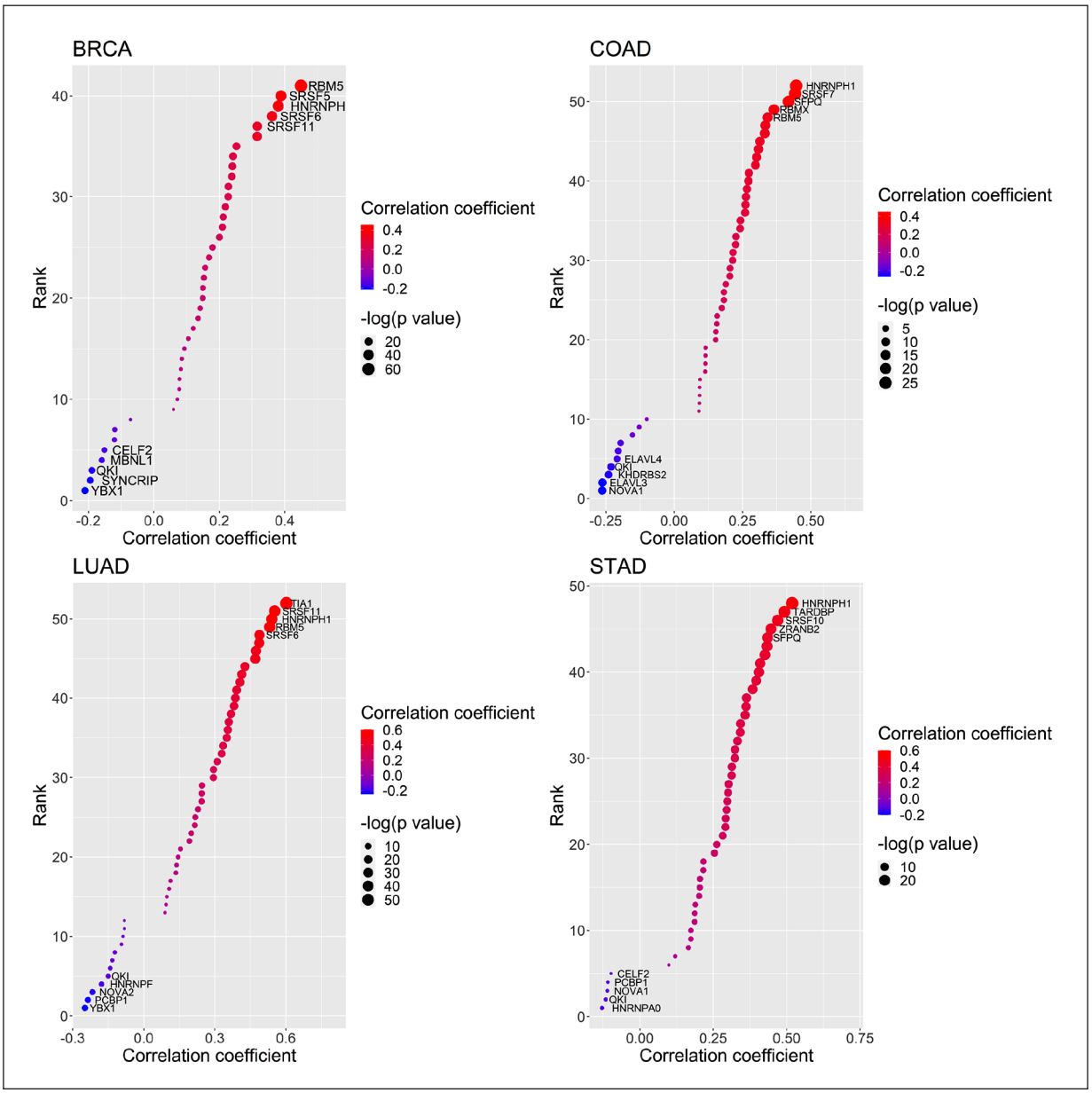

Figure 1. Correlation between the expression of METTL3 and each splicing factor in 4 types of cancer. Pearson's correlation coefficients between METTL3 and each splicing factor was calculated in breast invasive ductal carcinoma (BRCA), colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), and gastric adenocarcinoma (STAD). X-axis and dot color represent correlation coefficient values, and the size of dots represents $-\log_{10}(p\text{-value})$. Among the 64 splicing factors, only substantially correlated proteins are shown in the plot.

METTL3 expression in cancer cells. To understand the molecular mechanisms of SRSF11 in carcinogenesis, we performed GSEA using hallmark gene sets and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway gene sets based on SRSF11 expression (Figure 4, **Supplementary Figure 3**, and **Supplementary Table II**). Among 50 hallmark gene sets used for analysis, several carcinogenesis-related gene sets, such as 'Ultraviolet (UV) response up', 'Reactive oxygen species (ROS) pathway', 'Apoptosis', 'Glycolysis', 'p53 pathway', 'Epithelial mesenchymal transition', and 'Hypoxia' were substantially enriched in all cancer patients with decreased SRSF11 expression (Figure 4). In addition, 'Lysosome', 'Endocytosis', several metabolism-related pathways, and cancer-related pathways, such as 'Bladder cancer', 'Renal cell carcinoma', and 'Pancreatic

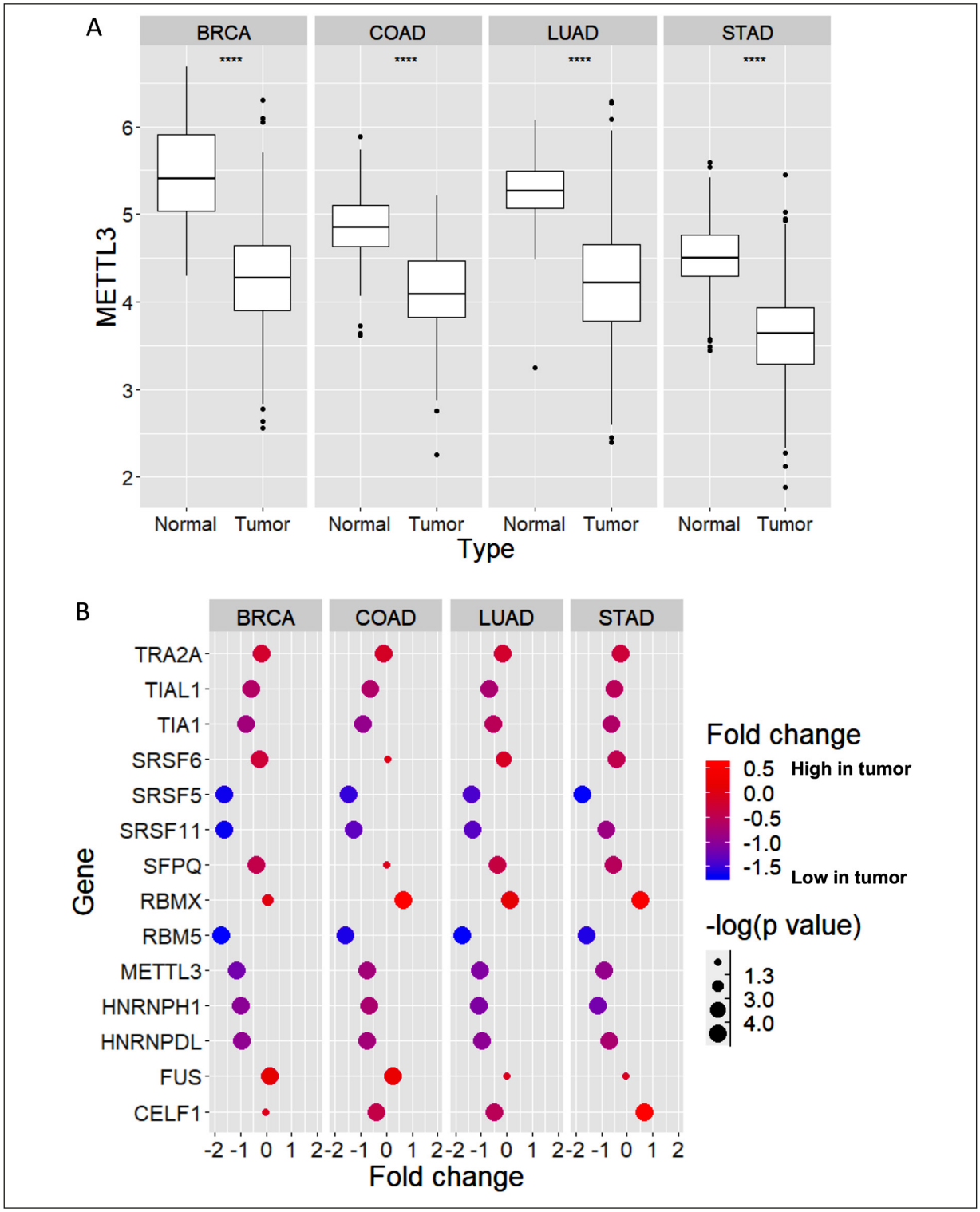

Figure 2. Expression levels of METTL3 and splicing factors in normal and tumor tissues. **A**, METTL3 mRNA expression levels [$\log_2$ (RPKM + 0.0001)] were analyzed in normal and cancer tissues using GTEX TCGA data. Wilcoxon rank-sum test was performed to compare the expressions owing to the relatively small number of normal tissue samples in TCGA data. Asterisks indicate significant differences (****$p < 0.0001$). **B**, Splicing factor expression levels were compared between normal and cancer tissues. X-axis and dot color represent fold change, and the size of each dot represents $-\log_{10}$ (p-value).

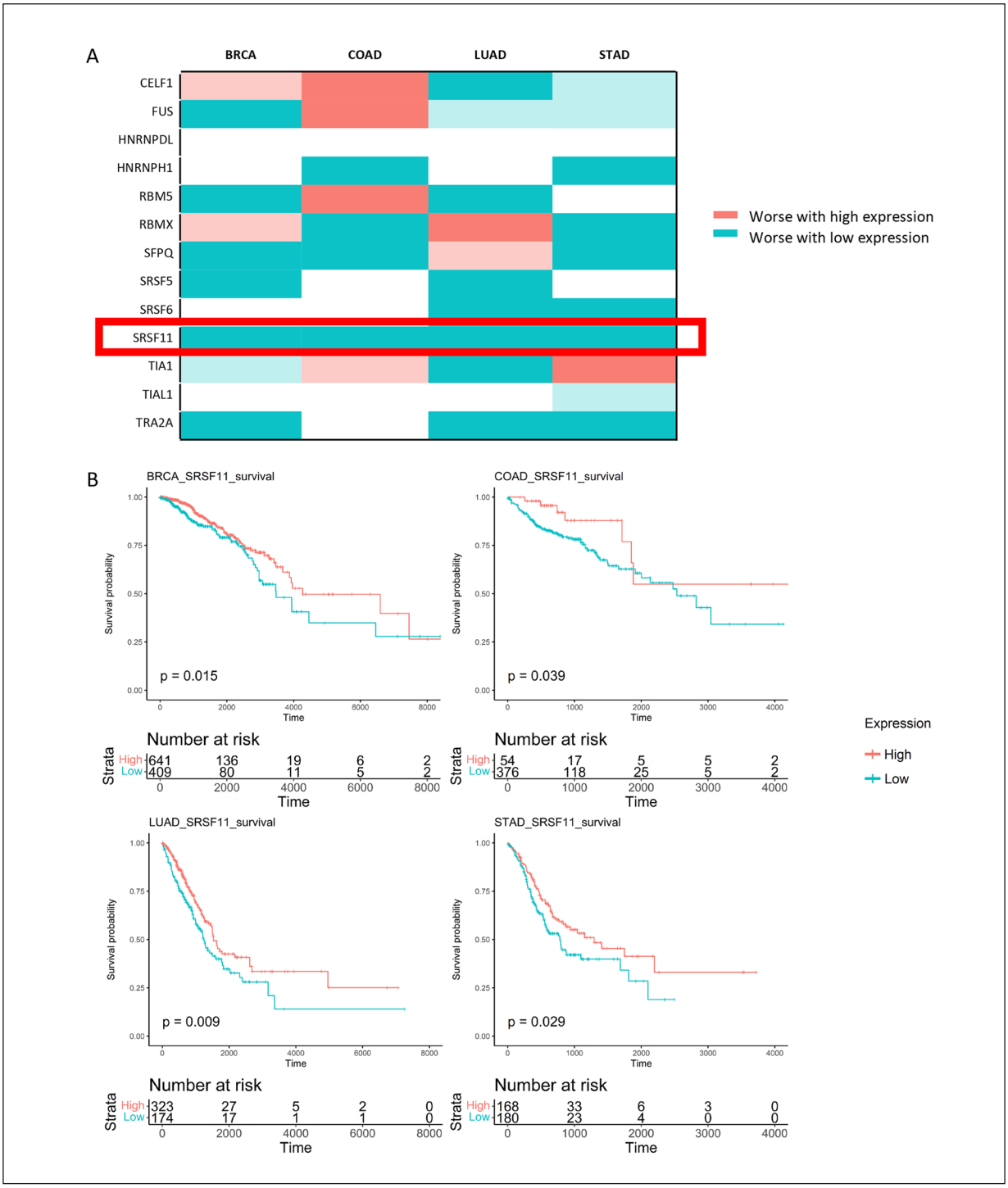

Figure 3. Survival analysis according to the expression of each splicing factor. **A**, For each gene, Kaplan-Meier plots and log rank tests were used to estimate the survival difference between patients with low and high gene expression. Blue indicates poor prognosis in patients with low gene expression, and red indicates for poor prognosis in patients with high gene expression. Color depth represents *p*-values from log rank test. The deeper ones have *p*-values lower than 0.05, and the lighter ones have *p*-values between 0.05 and 0.1. **B**, Kaplan-Meier plots according to SRSF11 expression level in breast invasive ductal carcinoma (BRCA), colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), and gastric adenocarcinoma (STAD).

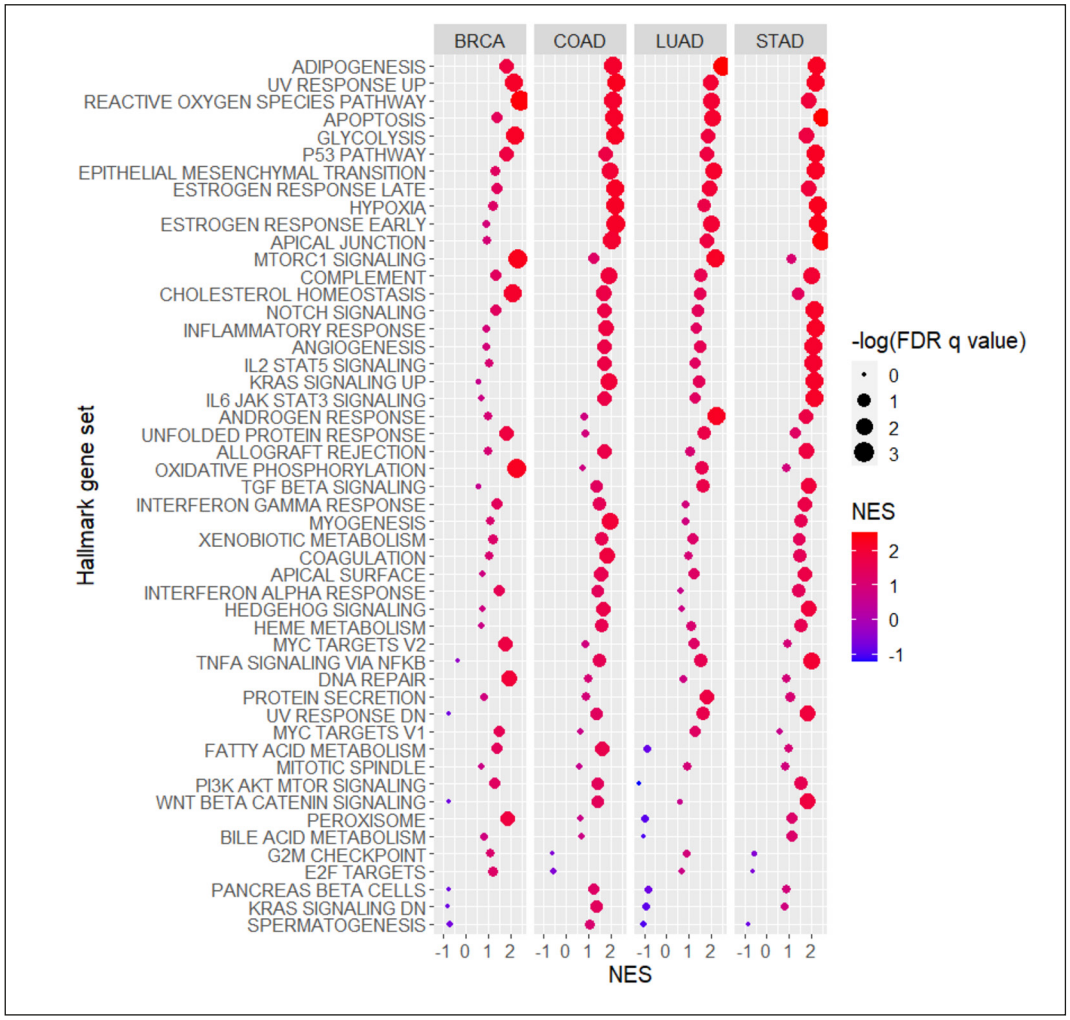

Figure 4. Hallmark gene set enrichment analysis according to the expression level of SRSF11. Gene set enrichment analysis (GSEA) was performed between the patient groups sorted by SRSF11 expression levels. Fifty hallmark gene sets were used for GSEA. Normal enrichment scores (NESs) are represented by dot color in X-axis and positive NESs represent enriched gene sets in patients with decreased *SRSF11* expression. Dot size represents $-\log_{10}$ (FDR *q*-value).

cancer' were enriched in patients with decreased SRSF11 expression based on KEGG pathway analysis (**Supplementary Figure 3**). These data suggest that *SRSF11* downregulation affects carcinogenesis by modulating gene expression related to the p53/apoptosis, inflammation/immune response, and UV/ROS stimulus response pathways.

Discussion

Proper RNA processing, including transcription initiation, splicing, and termination, is a major step in regulating gene expression. These processes are finely regulated by multiple factors, and mutation of each factor causes severe diseases, such as cancer[14-16]. As m^{6}A modification alters pre-mRNA splicing, we hypothesized that METTL3, which is the core component of the m^{6}A writer complex, affects the expression of splicing factors that regulate alternative splicing. To understand the general relationship between METTL3 and each splicing factor, we analyzed RNA expression and survival data using thousands of patient samples associated with four types of cancer: BRCA, COAD, LUAD, and STAD. The results showed that SRSF11 expression was substantially decreased in cancer cells with decreased METTL3 expression.

It is known that m^{6}A modification regulating genes function as either tumor suppressors or oncogenes depending on cancer types[22]. METTL14, another m^{6}A "writer" gene, was found to function as a tumor suppressor gene and oncogene in case of liver and breast cancer, respectively[23, 24]. We found that *METTL3* was downregulated in all four types of cancer tissues. Given these findings, it is reasonable to say that *METTL3* acts as a tumor suppressor gene in BRCA, COAD, LUAD, and STAD. In these four cancer types with decreased METTL3 expression, SRSF11 expression was also substantially decreased.

Among the 13 splicing factor expressions that showed a positive correlation with METTL3 expression, SRSF11 was the consistent prognostic factor in all four analyzed cancer types. Though SRSF11 is found to be a crucial splicing factor, little is known about it. Interestingly, *SRSF11* mutations and different isoforms were found in neurodevelopmental disorders and myelodysplastic syndromes, respectively[25, 26]. This finding suggests that aberrant splicing caused by *SRSF11* mutations and isoforms could affect the pathogenesis of those diseases. Similarly, our study showed that decreased SRSF11 expression could affect BRCA, COAD, LUAD, and STAD by decreasing the survival period of cancer patients and affecting the genes associated with the p53/apoptosis, inflammation/immune response, and UV/ROS stimulus response pathways.

Survival analysis data suggested that similar to METTL3, SRSF11 splicing factor behaves as a tumor suppressor in BRCA, COAD, LUAD, and STAD. Decreased SRSF11 expression was correlated with poor survival of patients with BRCA, COAD, LUAD, and STAD. This suggests that an effective method to increase SRSF11 expression could be used as a cancer treatment to increase the survival period of patients. One of the most common molecular mechanisms of METTL3-mediated regulation of SRSF11 is direct m^{6}A modification of *SRSF11* mRNA by METTL3. The m^{6}A target database (http://m6a2target.canceromics.org/#/)[27] showed that several m^{6}A reader proteins, including IGF2BP1/2/3, YTHDC1/2, and HNRNPC interact with *SRSF11* mRNA, as evaluated by eCLIP-seq. In particular, IGF2BP1/2/3 is one of the most common m^{6}A binding proteins that promote target mRNA stability[28], suggesting that METTL3 positively regulates SRSF11 in an m^{6}A/ IGF2BP1/2/3-dependent manner. However, further studies are necessary to elucidate the molecular mechanism of METTL3-regulated SRSF11 expression.

Conclusions

METTL3 expression is substantially correlated with the expression of several splicing factors, and among these factors, SRSF11 has a prognostic effect in cancer patients by regulating several cancer-related pathways. Our data suggest a novel molecular mechanism through which m^{6}A RNA methylation modulates alternative splicing by regulating splicing factor expressions.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2021R1C1C1005358, NRF-2021R1A2C3008021, and NRF-2018R1A5A2023879); Creative-Pioneering Researchers Program through Seoul National University (No. 800-20200510); the MD-Phd/Medical Scientist Training Program through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea; and 2021 BK21 FOUR Program of Pusan National University to J.-M. Oh.

Authors' Contribution

J.O., J.-M.O., and S.-Y.C. designed this study. J.O., did the relevant analyses. J.O., J.-M.O., and S.-Y.C. wrote the manuscript. All authors read and approved the final version of the manuscript.

Ethics Approval and Informed Consent

Not applicable.

References

1) Zaccara S, Ries RJ,Jaffrey SR. Reading, writing and erasing mRNA methylation. Nat Rev Mol Cell Biol 2019; 20: 608-624.
2) Han SH,Choe J. Diverse molecular functions of m(6)A mRNA modification in cancer. Exp Mol Med 2020; 52: 738-749.
3) Bokar JA, Shambaugh ME, Polayes D, Matera AG,Rottman FM. Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. RNA 1997; 3: 1233-1247.
4) Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, Jia G, Yu M, Lu Z, Deng X, Dai Q, Chen W,He C. A

METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol 2014; 10: 93-95.

5) Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, Adhikari S, Shi Y, Lv Y, Chen YS, Zhao X, Li A, Yang Y, Dahal U, Lou XM, Liu X, Huang J, Yuan WP, Zhu XF, Cheng T, Zhao YL, Wang X, Rendtlew Danielsen JM, Liu F,Yang YG. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res 2014; 24: 177-189.

6) Alarcon CR, Goodarzi H, Lee H, Liu X, Tavazoie S,Tavazoie SF. HNRNPA2B1 Is a Mediator of m(6)A-Dependent Nuclear RNA Processing Events. Cell 2015; 162: 1299-1308.

7) Du H, Zhao Y, He J, Zhang Y, Xi H, Liu M, Ma J,Wu L. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. Nat Commun 2016; 7: 12626.

8) Roundtree IA, Luo GZ, Zhang Z, Wang X, Zhou T, Cui Y, Sha J, Huang X, Guerrero L, Xie P, He E, Shen B,He C. YTHDC1 mediates nuclear export of N(6)-methyladenosine methylated mRNAs. Elife 2017; 6: e31311.

9) Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, Liu C,He C. YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA. Cell Res 2017; 27: 315-328.

10) Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H,He C. N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell 2015; 161: 1388-1399.

11) Zhang Z, Theler D, Kaminska KH, Hiller M, de la Grange P, Pudimat R, Rafalska I, Heinrich B, Bujnicki JM, Allain FH,Stamm S. The YTH domain is a novel RNA binding domain. J Biol Chem 2010; 285: 14701-14710.

12) Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG,He C. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011; 7: 885-887.

13) Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, Vagbo CB, Shi Y, Wang WL, Song SH, Lu Z, Bosmans RP, Dai Q, Hao YJ, Yang X, Zhao WM, Tong WM, Wang XJ, Bogdan F, Furu K, Fu Y, Jia G, Zhao X, Liu J, Krokan HE, Klungland A, Yang YG,He C. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 2013; 49: 18-29.

14) Ilagan JO, Ramakrishnan A, Hayes B, Murphy ME, Zebari AS, Bradley P,Bradley RK. U2AF1 mutations alter splice site recognition in hematological malignancies. Genome Res 2015; 25: 14-26.

15) Oh JM, Venters CC, Di C, Pinto AM, Wan L, Younis I, Cai Z, Arai C, So BR, Duan J,Dreyfuss G. U1 snRNP regulates cancer cell migration and invasion in vitro. Nat Commun 2020; 11: 1.

16) Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, Chalkidis G, Suzuki Y, Shiosaka M, Kawahata R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann WK, Miyawaki S, Sugano S, Haferlach C, Koeffler HP, Shih LY, Haferlach T, Chiba S, Nakauchi H, Miyano S,Ogawa S. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 2011; 478: 64-69.

17) Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R,Rechavi G. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 2012; 485: 201-206.

18) Zhao X, Yang Y, Sun BF, Shi Y, Yang X, Xiao W, Hao YJ, Ping XL, Chen YS, Wang WJ, Jin KX, Wang X, Huang CM, Fu Y, Ge XM, Song SH, Jeong HS, Yanagisawa H, Niu Y, Jia GF, Wu W, Tong WM, Okamoto A, He C, Rendtlew Danielsen JM, Wang XJ,Yang YG. FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. Cell Res 2014; 24: 1403-1419.

19) Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, Sun HY, Li A, Ping XL, Lai WY, Wang X, Ma HL, Huang CM, Yang Y, Huang N, Jiang GB, Wang HL, Zhou Q, Wang XJ, Zhao YL,Yang YG. Nuclear m(6)A Reader YTHDC1 Regulates mRNA Splicing. Mol Cell 2016; 61: 507-519.

20) Giulietti M, Piva F, D'Antonio M, D'Onorio De Meo P, Paoletti D, Castrignano T, D'Erchia AM, Picardi E, Zambelli F, Principato G, Pavesi G,Pesole G. SpliceAid-F: a database of human splicing factors and their RNA-binding sites. Nucleic Acids Res 2013; 41: D125-131.

21) Oh J, Hwa C, Jang D, Shin S, Lee SJ, Kim J, Lee SE, Jung HR, Oh Y, Jang G, Kwon O, An JY,Cho SY. Augmentation of the RNA m6A reader signature is associated with poor survival by enhancing cell proliferation and EMT across cancer types. Exp Mol Med 2022; 54: 906-921.

22) Ji JW, Zhang YD, Lai YJ,Huang CG. Mettl3 regulates the proliferation, migration and invasion of glioma cells by inhibiting PI3K/Akt signaling pathway. Eur Rev Med Pharmacol Sci 2020; 24: 3818-3828.

23) Ma JZ, Yang F, Zhou CC, Liu F, Yuan JH, Wang F, Wang TT, Xu QG, Zhou WP,Sun SH. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N(6) -methyladenosine-dependent primary MicroRNA processing. Hepatology 2017; 65: 529-543.

24) Sun T, Wu Z, Wang X, Wang Y, Hu X, Qin W, Lu S, Xu D, Wu Y, Chen Q, Ding X, Guo H, Li Y, Wang Y, Fu B, Yao W, Wei M,Wu H. LNC942 promoting METTL14-mediated m(6)A methylation in breast cancer cell proliferation and progression. Oncogene 2020; 39: 5358-5372.

25) RK CY, Merico D, Bookman M, J LH, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N,

Bingham J, Wang Z, Pellecchia G, Buchanan JA, Walker S, Marshall CR, Uddin M, Zarrei M, Deneault E, D'Abate L, Chan AJ, Koyanagi S, Paton T, Pereira SL, Hoang N, Engchuan W, Higginbotham EJ, Ho K, Lamoureux S, Li W, MacDonald JR, Nalpathamkalam T, Sung WW, Tsoi FJ, Wei J, Xu L, Tasse AM, Kirby E, Van Etten W, Twigger S, Roberts W, Drmic I, Jilderda S, Modi BM, Kellam B, Szego M, Cytrynbaum C, Weksberg R, Zwaigenbaum L, Woodbury-Smith M, Brian J, Senman L, Iaboni A, Doyle-Thomas K, Thompson A, Chrysler C, Leef J, Savion-Lemieux T, Smith IM, Liu X, Nicolson R, Seifer V, Fedele A, Cook EH, Dager S, Estes A, Gallagher L, Malow BA, Parr JR, Spence SJ, Vorstman J, Frey BJ, Robinson JT, Strug LJ, Fernandez BA, Elsabbagh M, Carter MT, Hallmayer J, Knoppers BM, Anagnostou E, Szatmari P, Ring RH, Glazer D, Pletcher MT,Scherer SW. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat Neurosci 2017; 20: 602-611.

26) Wang H, Wen J, Chang CC,Zhou X. Discovering transcription and splicing networks in myelodysplastic syndromes. PLoS One 2013; 8: e79118.

27) Deng S, Zhang H, Zhu K, Li X, Ye Y, Li R, Liu X, Lin D, Zuo Z,Zheng J. M6A2Target: a comprehensive database for targets of m6A writers, erasers and readers. Brief Bioinform 2021; 22: bbaa055.

28) Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, Zhao BS, Mesquita A, Liu C, Yuan CL, Hu YC, Huttelmaier S, Skibbe JR, Su R, Deng X, Dong L, Sun M, Li C, Nachtergaele S, Wang Y, Hu C, Ferchen K, Greis KD, Jiang X, Wei M, Qu L, Guan JL, He C, Yang J,Chen J. Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat Cell Biol 2018; 20: 285-295.