ANTICANCER RESEARCH *40*: 2941-2946 (2020)
doi:10.21873/anticanres.14272

SF3B4 Plays an Oncogenic Role in Esophageal Squamous Cell Carcinoma

SHINYA KIDOGAMI[1,2], TOMOHIRO IGUCHI[1], KUNIAKI SATO[1], YUKIHIRO YOSHIKAWA[1,2], QUINGJANG HU[1], SHO NAMBARA[1], HISATERU KOMATSU[1,2], MASAMI UEDA[1,2], YOSUKE KURODA[1], TAKAAKI MASUDA[1], MASAKI MORI[2], YUICHIRO DOKI[2] and KOSHI MIMORI[1]

[1]*Department of Surgery, Kyushu University Beppu Hospital, Oita, Japan;*
[2]*Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan*

Abstract. *Background/Aim: The spliceosome pathway, including Splicing Factor 3b Subunit 4 (SF3B4), plays an important role in carcinogenesis and progression in various cancers; however, the clinical relevance of SF3B4 in esophageal squamous cell carcinoma (ESCC) remains unknown. Patients and Methods: SF3B4 expression was evaluated by real-time reverse transcription polymerase chain reaction in 80 ESCC patients. In order to explore the mechanism of SF3B4 in ESCC, the mRNA expression and copy number of SF3B4 were obtained from TCGA and we also implemented gene set enrichment analysis (GSEA). Results: The high SF3B4 expression group (n=33) showed significantly more lymphatic permeation and poorer prognosis than the low SF3B4 expression group (n=47). GSEA revealed that high SF3B4 expression was correlated with genes associated with the transcription factor E2F and the G_2/M checkpoint. SF3B4 expression was positively correlated with SF3B4 DNA copy number. Conclusion: Over-expression of SF3B4 may play a crucial role in the lymphatic progression of ESCC.*

Esophageal squamous cell carcinoma (ESCC) is one of the most frequent types of malignant cancer in East Asian countries, including Japan (1). Recent advancements in multidisciplinary therapy have improved clinical outcomes to some extent. However, the 5-year survival frequency of ESCC patients is still only 30-40% because of the development of lymph node metastasis and subsequent tumor invasion into adjacent critical organs (2-6). It is extremely important to clarify the molecular mechanisms underlying the progression of ESCC to improve patient outcome.

Correspondence to: Koshi Mimori, MD, Ph.D., Department of Surgery, Kyushu University, Beppu Hospital, 4546 Tsurumihara, Beppu 874-0838, Japan. Tel: +81 977271650, Fax: +81 977271651, e-mail: kmimori@beppu.kyushu-u.ac.jp

Key Words: SF3B4, esophageal squamous cell carcinoma, poor prognostic biomarker.

The spliceosome is composed of 5 small nuclear ribonucleoproteins (snRNPs) – U1, U2, U4, U5 and U6 – and multiple other proteins play an important role in the splicing process (7, 8). The involvement of alternative splicing factors has been reported in various diseases, including cancers (9). As a result, dysfunction of alternative splicing and aberrant production of specific splicing variants promote carcinogenesis, progression and chemo-resistance (10-12). Recently, bioinformatic studies have shown the contribution of the spliceosomal pathway to the progression of various cancers (13-17). In ESCC, upregulation of *miR-196a* was observed and target pathway analysis showed a strong association with the spliceosome pathway (17).

Splicing factor B subunit 4 (SF3B4) is a component of the U2 pre-mRNA spliceosomal complex. Loss of function mutations in *SF3B4* are the major cause of Nager syndrome (18-20). Recently, Shen *et al.* have reported an intriguing finding concerning the early carcinogenic role of the *SF3B4* gene in hepatoma. The upregulated SF3B4 accelerates the production of alternative splicing variants of the Kruppel-like factor 4 (*KLF4)* gene to be a nonfunctional skipped exon transcripts. That dysfunction leads to the inactivation of the p27 (*CDKN1B*) gene, which is involved in the activation of the *SNAI2 gene,* an epithelial to mesenchymal transition-related gene (21). We have also reported that *SF3B4* is upregulated in hepatocellular carcinoma (HCC) and is associated with the prognosis of HCC patients (22). However, the relevance of *SF3B4* in ESCC is unknown. Thus, in the current study, we aimed to clarify the clinicopathological and prognostic significance of *SF3B4* expression in ESCC.

Patients and Methods

Patients. Primary ESCC samples and their corresponding adjacent epithelia were obtained from 80 patients who underwent esophagostomy at our institute (Kyushu University Beppu Hospital) and our affiliated hospitals between1998 and 2012 (Kyushu dataset). All patients had a histological diagnosis of ESCC and were closely followed at 3-month intervals after surgery. The median follow-up period was 3.9 years. All

patients were treated in accordance with the Japan Esophageal Society Guidelines for the treatment of ESCC. Informed consent was obtained from all patients, and the Institutional Review Board of our university approved this study. Following collection, all resected ESCC and the adjacent normal esophageal epithelial samples were immediately frozen in liquid nitrogen and stored at –80˚C until RNA extraction. All clinicopathological data, including patients' age, sex, histological grade, tumor depth of invasion, location, lymph node metastasis, lymphatic invasion and vascular invasion were obtained from the clinical and pathological records.

RNA preparation, reverse transcription (RT) reaction and quantitative polymerase chain reaction (qPCR). Total RNA from esophageal cancer or adjacent normal epithelial were extracted by the modified Acid Guanidinium Phenol Chloroform (AGPC) method using ISOGEN (Nippon Gene, Tokyo, Japan). RT was performed using 8 μg of total RNA with M-MLV reverse transcriptase according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA).

Quantitative polymerase chain reaction (qPCR). qPCR was performed in a LightCycler 480 instrument (Roche Applied Science, Basel, Switzerland) using a LightCycler 480 Probes Master kit (Roche Applied Science) as described previously (22). In brief, PCR primer sequences for human *SF3B4* were as follows: sense, 5'-AGACGGCGGGATCTCTTT-3'; antisense, 5'-CACGTACAC AGTGGCATCCT-3'. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) primers, which served as the internal control to normalize the expression level of *SF3B4*, were as follows: sense, 5'-TTGGTATCGTGGAAGGACTCTCA-3'; antisense, 5'-TGTCA TATTTGGCAGGTT-3'. The amplification conditions were as follows: 10 min at 95˚C, followed by 45 cycles of 10 sec at 95˚C and 30 sec at 60˚C. The expression levels were expressed relative to the expression levels of Human Universal Reference Total RNA (Clontech, Palo Alto, CA, USA).

Public clinical dataset. We obtained paired *SF3B4* expression profiles and survival data of 86 available ESCC cases from The Cancer Genome Atlas (TCGA) of the Broad Institute's Firehose (https://gdac.broadinstitute.org). Copy number data for 90 ESCC cases were also obtained from TCGA.

Gene set enrichment analysis (GSEA). We acquired ESCC expression profiles from the National Center for Biotechnology Information gene expression omnibus database (accession code GSE2533) and analyzed the correlations between *SF3B4* expression and previously annotated gene expression signatures by applying GSEA (23, 24).

Statistical analysis. Either the X^2 test or Fisher's exact test was used for comparisons between *SF3B4* expression and clinicopathological findings. Survival curves were calculated by the Kaplan–Meier method and analyzed by the log-rank test. A comparison of *SF3B4* expression in ESCC and normal epithelial tissue was evaluated using the Mann-Whitney's *U*-test. These results were obtained using R version 3.1.1 [R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org]. *p*-Values less than 0.05 were considered statistically significant.

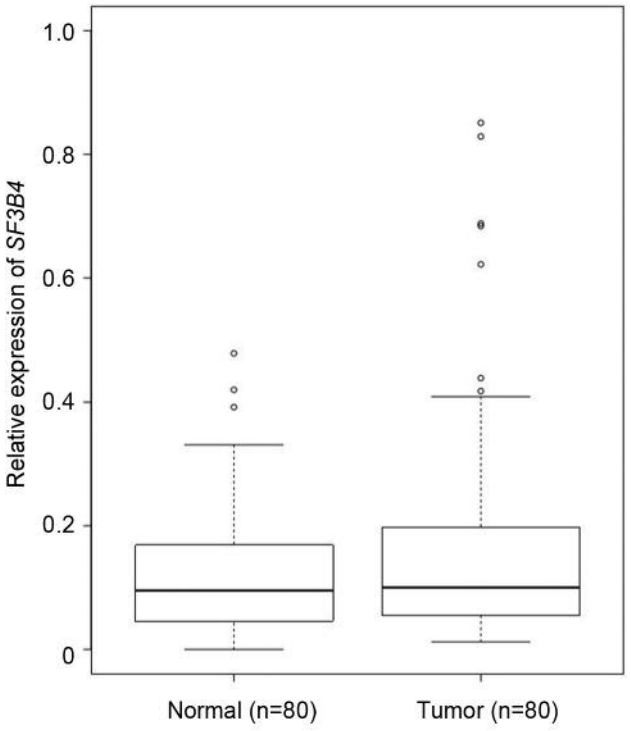

Figure 1. *Comparison of SF3B4 expression levels in ESCC and adjacent normal esophageal epithelial tissue. SF3B4 expression in ESCC was significantly higher than that in the normal esophageal epithelial tissue (p=0.03).*

Results

Comparison of SF3B4 expression levels between ESCC and normal epithelial tissue. We compared the *SF3B4* expression levels between ESCC and the adjacent normal epithelial tissue by qPCR. *SF3B4* expression was significantly higher in ESCC tumor tissues than in normal epithelial tissue (p=0.03; Figure 1).

Prognostic relevance of SF3B4 expression in ESCC. Next, to estimate the clinical significance of *SF3B4* expression in ESCC, we divided the 80 patients in the Kyushu dataset into two groups: a high *SF3B4* expression group and a low *SF3B4* expression group, according to the ratio of *SF3B4* expression in tumor tissue to that of normal epithelial tissue (T/N). The cut-off line was set to T/N=2. Patients in the *SF3B4* high expression group had significantly poorer outcomes than those in the *SF3B4* low expression group (p<0.01; Figure 2A). In addition, TCGA dataset also revealed that the high *SF3B4* expression group had significantly poorer overall survival than the low *SF3B4* expression group (p=0.04; Figure 2B).

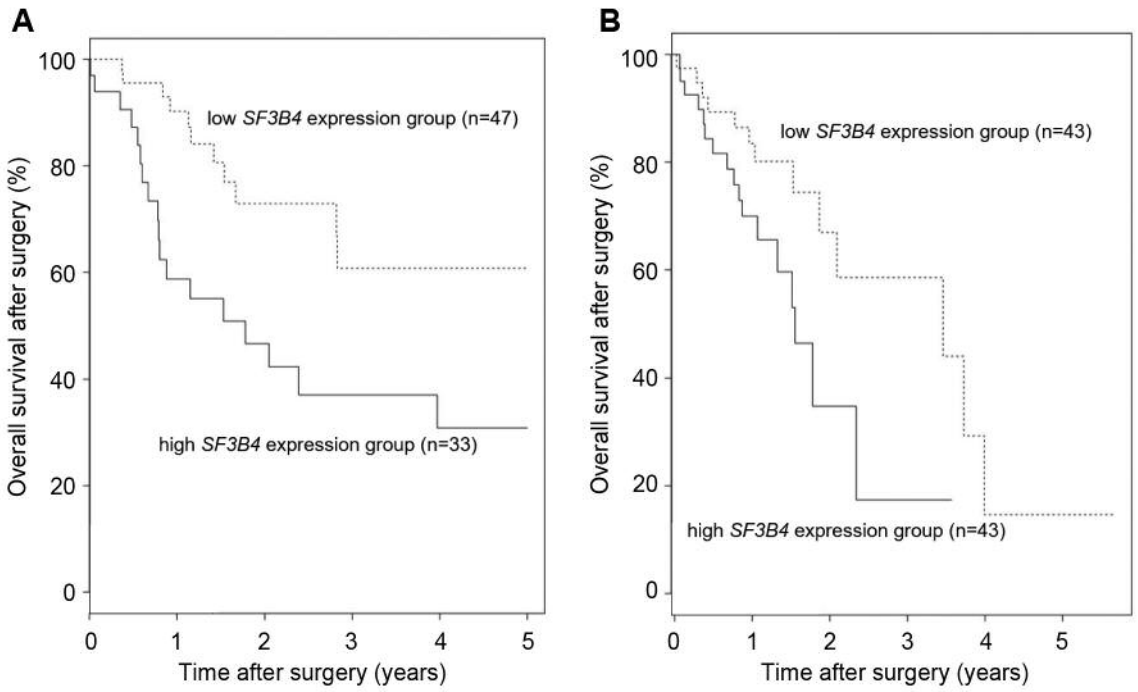

Figure 2. *Kaplan–Meier curves for the high and low SF3B4 expression groups. The cut-off lines are T/N=2.0 (A) in the Kyushu dataset and the median (B) in the TCGA dataset. The high SF3B4 group had significantly poorer outcomes than the low SF3B4 group in the Kyushu dataset (A) and TCGA dataset (B) (p<0.01 and p=0.04, respectively). Dotted line, low SF3B4 expression group; solid line, high SF3B4 expression group.*

Table I. *Comparative Analysis of Clinicopathological Findings in the SF3B4 high and low expression groups.*

Factors	High *SF3B4* expression group (n=47)	High *SF3B4* expression group (n=33)	*p*-Value
Age (year: mean ± SD)	65.0±8.7	63.3±8.3	0.71
Gender (male/female)	44/3	28/5	0.26
Location (Ce+Ut/Mt/Lt+Ae/NA)	2/21/22/2	3/14/8/8	0.31
Histological grade (G1/G2/G3/NA)	20/15/10/2	8/13/6/6	0.41
Tfactor (1/2/3/4)	6/5/32/4	4/4/20/5	0.79
Lymph node metastasis (0/1/2/3,4)	13/9/18/7	10/10/5/8	0.13
Lymphatic invasion (0/1/2/NA)	11/22/12/2	2/13/17/1	0.03
Vascular invasion (0/1/2/NA)	9/28/8/2	2/18/12/1	0.08

Ce: Cervical esophagus; Ut: upper thoracic esophagus; Mt: middle thoracic esophagus; Lt: lower thoracic esophagus; Ae: abdominal esophagus; SD: standard deviation; NA: not available.

Correlations between SF3B4 expression and clinicopathological factors. We examined the correlations between *SF3B4* expression level and clinicopathological factors of patients in the Kyushu dataset (Table I). Lymphatic invasion was more frequently observed in the high *SF3B4* expression group than in the low *SF3B4* expression group (p=0.03). With respect to other clinicopathological factors, there were no significant differences between the 2 groups.

Expression of SF3B4 correlated with the cell cycle in ESCC. We investigated the function of *SF3B4* in ESCC by applying GSEA to ESCC cases (GSE2533). The results showed that *SF3B4* expression was positively correlated with E2F target genes' expression (p<0.01; Figure 3A). In addition, *SF3B4* expression was positively correlated with the expression of genes associated with the G_2/M checkpoint (p<0.01; Figure 3B).

Correlation between SF3B4 DNA copy number variation and SF3B4 expression. To examine the influence of DNA copy number variation on *SF3B4*, we analyzed the correlation between the copy number and expression levels of *SF3B4* in

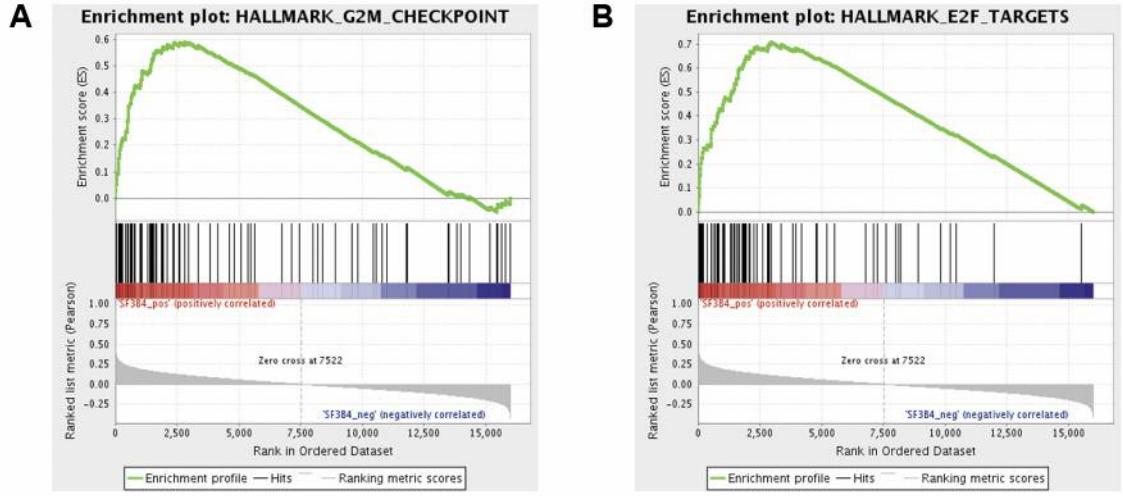

Figure 3. *Gene set enrichment analysis (GSEA): Enriched gene sets for ESCC patients with high SF3B4 expression; HALLMARK_G2M_CHECKPOINT (A) and HALLMARK_E2F_CHECKPOINT (B). SF3B4 expression was positively correlated with genes associated with E2F target genes (A) and G2/M checkpoint genes (B).*

TCGA dataset. *SF3B4* expression was positively correlated with *SF3B4* DNA copy number (R=0.47, p<0.01; Figure 4).

Discussion

Few studies have examined the biological role of *SF3B4* in solid cancers. In our previous study, *SF3B4* overexpression was a significant indicator of malignant outcomes in HCC (22), while another study showed that *SF3B4* had a suppressive role in pancreatic cancer (25). Although, it has been reported that the spliceosome pathway is strongly involved in carcinogenesis and development via microRNA 196a in ESCC (17), the relationship between ESCC and *SF3B4* has not been examined. Here we provide the first description of the clinicopathological significance of *SF3B4* in ESCC through analysis of the Kyushu dataset and a public dataset. We showed that the expression of *SF3B4* was upregulated in ESCC tissues compared with corresponding normal tissues. Moreover, elevated expression was associated with poor prognoses in ESCC, suggesting that *SF3B4* may act as an oncogene in ESCC.

It is not clear why *SF3B4* functions as an oncogene in ESCC. Shen et al. have reported that *SF3B4* selectively modulated the epithelial-mesenchymal transition (EMT) by enhancing *SNAI2* in HCC cells (21). EMT is significantly involved in carcinogenesis and progression of ESCC (26, 27). Our clinicopathological analysis revealed that *SF3B4* expression was involved in lymphatic invasion and poor prognosis in ESCC. The molecular mechanism of lymphovascular invasion is closely related to EMT (28). Warzecha *et al*. have reported that changes in selective RNA

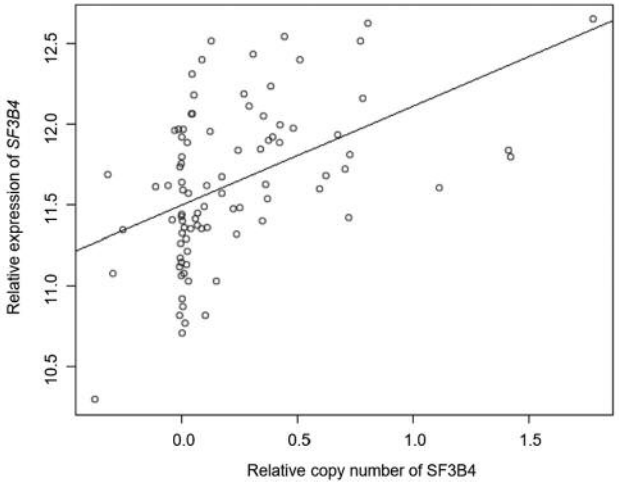

Figure 4. *Relationship between copy number and expression levels of SF3B4. SF3B4 expression was positively correlated with SF3B4 DNA copy number (R=0.47, p<0.01).*

splicing occur in specific genes in the EMT process as they switch from an epithelial system-specific isoform to a mesenchymal-specific isoform (29, 30).

SF3B4 triggers the production of alternative splicing variants of the tumor suppressor Kruppel-like factor 4 (KLF4), rendering its transcripts nonfunctional. It therefore promotes cell cycle progression through inactivation of p27Kip (21). Our GSEA study revealed that *SF3B4* modulates the genes encoding cell cycle-related targets of E2F transcription factors. The E2F family is a target of

Retinoblastoma (RB) proteins and plays an important role in cell cycle regulation, especially at the G_1/S interphase (31). Thus, aberrant *SF3B4* expression may regulate the cell cycle. We also showed that genes associated with the G_2/M checkpoint were also associated with *SF3B4* expression. Consistent with our previous report (22), a positive correlation between *SF3B4* expression and DNA copy number was confirmed. In acute lymphoblastic leukemia and Burkitt's lymphoma, a duplicated copy of the chromosomal region containing *SF3B4* was detected by FISH (32). Chromosomal aberrations may play a role in *SF3B4* regulation. The detailed molecular mechanisms remain elusive and further analysis is required.

In conclusion, *SF3B4* plays an important oncogenic role in the progression of ESCC. *SF3B4* could be a therapeutic target, as well as novel prognostic factor in ESCC.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Shinya Kidogami: study concept and design, drafting of manuscript; Tomohiro Iguchi: study concept and critical revision of the manuscript; Kuniaki Sato: statistical analysis; Yukihiro Yoshikawa: data collection; Quingjang Hu: statistical analysis, Sho Nambara: experiment; Hisateru Komatsu: data collection; Masami Ueda: experiment; Yosuke Kuroda: data collection; Takaaki Masuda: study concept; Masaki Mori: critical revision of the manuscript; Yuichiro Doki; critical revision of the manuscript; Koshi Mimori: final approval of the manuscript.

Acknowledgements

This work was supported by the following grants and foundations: Grants-in-Aid for Scientific Research of MEXT (24008081, 25430111, 25461953, 25861199, 25861200, 24592005 and 21229015); Funding Program for Next Generation World-Leading Researchers (LS094); Grants-in-Aid for Scientific Research on Innovative Areas of MEXT "Systems Cancer Research" (4201); The MEXT Strategic Programs on Innovative Research "Supercomputational Life Science." This research used computational resources of the K computer provided by the RIKEN Advanced Institute for Computational Science through the HPCI System Research project (Project ID: hp140230). Computation time was also provided by the Supercomputer System, Human Genome Center, Institute of Medical Science, University of Tokyo (http://sc.hgc.jp/shirokane.html). The OITA Cancer Research Foundation 2014. Grant-in-Aid for Scientific Research of Ministry of Health, Labor and Welfare (MHLW) (14524362 and 14525288). Clinical samples and corresponding clinical information were provided by Oita Red Cross Hospital (Oita, Japan), Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital (Hiroshima, Japan) and Iizuka Hospital (Fukuoka, Japan). The Authors appreciate the technical support of Ms. Kazumi Oda, Michiko Kasagi, Sachiko Sakuma and Tomoko Kawano.

References

1 Shimada H, Kitabayashi H, Nabeya Y, Okazumi S, Matsubara H, Funami Y, Miyazawa Y, Shiratori T, Uno T, Itoh H and Ochiai T: Treatment response and prognosis of patients after recurrence of esophageal cancer. Surgery *133*: 24-31, 2003. PMID: 12563234. DOI: 10.1067/msy.2003.31

2 Enzinger PC and Mayer RJ: Esophageal cancer. N Engl J Med *349*: 2241-2252, 2003. PMID: 25539106. DOI: 10.1056/NEJMra1314530

3 Kleinberg L and Forastiere AA: Chemoradiation in the management of esophageal cancer. J Clin Oncol *25*: 4110-4117, 2007. PMID: 17827461. DOI: 10.1200/JCO.2007.12.0881

4 Nakamura T, Ota M, Narumiya K, Sato T, Ohki T, Yamamoto M and Mitsuhashi N: Multimodal treatment for lymph node recurrence of esophageal carcinoma after curative resection. Ann Surg Oncol *15*: 2451-2457, 2008. PMID: 18592318. DOI: 10.1245/s10434-008-0016-x

5 Medical Research Council Oesophageal Cancer Working Group: Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. Lancet *359*: 1727-1733, 2002. PMID: 12049861. DOI: 10.1016/S0140-6736(02)08651-8

6 Ando N, Iizuka T, Kakegawa T, Isono K, Watanabe H, Ide H, Tanaka O, Shinoda M, Takiyama W, Arimori M, Ishida K and Tsugane S: A randomized trial of surgery with and without chemotherapy for localized squamous carcinoma of the thoracic esophagus: the Japan Clinical Oncology Group Study. J Thorac Cardiovasc Surg *114*: 205-209, 1997. PMID: 9270637. DOI: 10.1016/S0022-5223(97)70146-6

7 Maniatis T and Tasic B: Alternative pre-mRNA splicing and proteome expansion in metazoans. Nature *418*: 263-243, 2002. PMID: 12110900. DOI: 10.1038/418236a

8 Matera AG and Wang Z: A day in the life of the spliceosome. Nat Rev Mol Cell Biol *15*: 108-121, 2014. PMID: 24452469. DOI: 10.1038/nrm3742

9 Wang GS and Cooper TA: Splicing in disease: disruption of the splicing code and the decoding machinery. Nat Rev Genet *8*: 749-761, 2007. PMID: 17726481. DOI: 10.1038/nrg2164

10 Grosso AR, Martins S and Carmo-Fonseca M: The emerging role of splicing factors in cancer. EMBO Rep *9*: 1087-1093, 2008. PMID: 18846105 .DOI: 10.1038/embor.2008.189

11 Matlin AJ, Clark F and Smith CW: Understanding alternative splicing: towards a cellular code. Nat Rev Mol Cell Biol *6*: 386-398, 2005. PMID: 15956978. DOI: 10.1038/nrm1645

12 Kim E, Goren A and Ast G: Insights into the connection between cancer and alternative splicing. Trends Genet *24*: 7-10, 2008. PMID: 18054115. DOI: 10.1016/j.tig.2007.10.001

13 Wang Y, Li J, Chen J, Liu L, Peng Z, Ding J and Ding K: From cirrhosis to hepatocellular carcinoma in HCV-infected patients: genes involved in tumor progression. Eur Rev Med Pharmacol Sci *16*: 995-1000, 2012. PMID: 22913147.

14 Wong YH, Wu CC, Lin CL, Chen TS, Chang TH and Chen BS: Applying NGS data to find evolutionary network biomarkers from the early and late stages of hepatocellular carcinoma. Biomed Res Int *2015*: 391475, 2015. PMID: 26366411. DOI: 10.1155/2015/391475

15 Tian M, Cheng H, Wang Z, Su N, Liu Z, Sun C, Zhen B, Hong X, Xue Y and Xu P: Phosphoproteomic analysis of the highly-metastatic hepatocellular carcinoma cell line, MHCC97-H. Int J

Mol Sci *16*: 4209-4225, 2015. PMID: 25690035. DOI: 10.3390/ijms16024209

16 Xu W, Huang H, Yu L and Cao L: Meta-analysis of gene expression profiles indicates genes in spliceosome pathway are up-regulated in hepatocellular carcinoma (HCC). Med Oncol *32*: 96, 2015. PMID: 25731616. DOI: 10.1007/s12032-014-0425-6

17 Fendereski M, Zia MF, Shafiee M, Safari F, Saneie MH and Tavassoli M: MicroRNA-196a as a potential diagnostic biomarker for esophageal squamous cell carcinoma. Cancer Invest *35*: 78-84, 2017. PMID: 28095062. DOI: 10.1080/07357907.2016.1254228

18 Bernier FP, Calueriu O, Ng S, Schwartzentruber J, Buckingham KJ, Innes AM, Jabs EW, Innis JW, Schuette JL, Gorski JL, Byers PH, Andelfinger G, Siu V, Lauzon J, Fernandez BA, McMillin M, Scott RH, Racher H; FORGE Canada Consortium, Majewski J, Nickerson DA, Shendure J, Bamshad MJ and Parboosingh JS: Haploinsufficiency of SF3B4, a component of the pre-mRNA spliceosomal complex, causes Nager syndrome. Am J Hum Genet *90*: 925-933, 2012. PMID: 22541558. DOI: 10.1016/j.ajhg.2012.04.004

19 Lehalle D, Wieczorek D, Zechi-Ceide RM, Passos-Bueno MR, Lyonnet S, Amiel J and Gordon CT: A review of craniofacial disorders caused by spliceosomal defects. Clin Genet *88*: 405-415, 2015. PMID: 25865758. DOI: 10.1111/cge.12596

20 Czeschik JC, Voigt C, Alanay Y, Albrecht B, Avci S, Fitzpatrick D, Goudie DR, Hehr U, Hoogeboom AJ, Kayserili H, Simsek-Kiper PO, Klein-Hitpass L, Kuechler A, López-González V, Martin M, Rahmann S, Schweiger B, Splitt M, Wollnik B, Lüdecke HJ, Zeschnigk M and Wieczorek D: Clinical and mutation data in 12 patients with the clinical diagnosis of Nager syndrome. Hum Genet *132*: 885-898, 2013. PMID: 23568615. DOI: 10.1007/s00439-013-1295-2

21 Shen Q, Eun JW, Lee K, Kim HS, Yang HD, Kim SY, Lee EK, Kim T, Kang K, Kim S, Min DH, Oh SN, Lee YJ, Moon H, Ro SW, Park WS, Lee JY and Nam SW: Barrier to autointegration factor 1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3, and splicing factor 3b subunit 4 as early-stage cancer decision markers and drivers of hepatocellular carcinoma. Hepatology *67*: 1360-1377, 2018. PMID: 29059470. DOI:10.1002/hep.29606

22 Iguchi T, Komatsu H, Masuda T, Nambara S, Kidogami S, Ogawa Y, Hu Q, Saito T, Hirata H, Sakimura S, Uchi R, Hayashi N, Ito S, Eguchi H, Sugimachi K, Maehara Y and Mimori K: Increased copy number of the gene encoding SF3B4 indicates poor prognosis in hepatocellular carcinoma. Anticancer Res *36*: 2139-2144, 2016. PMID: 27127115.

23 Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP: Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA *102*: 15545-15550, 2005. PMID: 16199517. DOI: 10.1073/pnas.0506580102

24 Wei G, Luo H, Sun Y, Li J, Tian L, Liu W, Liu L, Luo J, He J and Chen R: Transcriptome profiling of esophageal squamous cell carcinoma reveals a long noncoding RNA acting as a tumor suppressor. Oncotarget *6*: 17065-17080, 2015. PMID: 26158411. DOI: 10.18632/oncotarget.4185

25 Zhou W, Ma N, Jiang H, Rong Y, Deng Y, Feng Y, Zhu H, Kuang T, Lou W, Xie D and Wang D: SF3B4 is decreased in pancreatic cancer and inhibits the growth and migration of cancer cells. Tumour Biol *39*: 1010428317695913, 2017. PMID: 28351319. DOI: 10.1177/1010428317695913

26 Sudo T, Iwaya T, Nishida N, Sawada G, Takahashi Y, Ishibashi M, Shibata K, Fujita H, Shirouzu K, Mori M and Mimori K: Expression of mesenchymal markers vimentin and fibronectin: the clinical significance in esophageal squamous cell carcinoma. Ann Surg Oncol *20*: S324-335, 2013. PMID: 22644514. DOI: 10.1245/s10434-012-2418-z

27 Iguchi T, Ueda M, Masuda T, Nambara S, Kidogami S, Komatsu H, Sato K, Tobo T, Ogawa Y, Hu Q, Saito T, Hirata H, Sakimura S, Uchi R, Hayashi N, Ito S, Eguchi H, Sugimachi K, Maehara Y and Mimori K: Identification of UHRF2 as a negative regulator of epithelial-mesenchymal transition and its clinical significance in esophageal squamous cell carcinoma. Oncology *95*: 179-187, 2018. PMID: 29909415. DOI: 10.1159/000488860

28 May M, Brookman-May S, Burger M, Koch S, Otto W, Bründl J, Albrecht K and Denzinger S: A switch from epithelial to mesenchymal properties correlates with lymphovascular invasion in squamous cell carcinoma of the penis. Pathol Res Pract *211*: 641-645, 2015. PMID: 26092595. DOI: 10.1016/j.prp.2015.05.007

29 Warzecha CC, Jiang P, Amirikian K, Dittmar KA, Lu H, Shen S, Guo W, Xing Y and Carstens RP: An ESRP-regulated splicing programme is abrogated during the epithelial-mesenchymal transition. Embo J *29*: 3286-3300, 2010. PMID: 20711167. DOI: 10.1038/emboj.2010.195

30 Warzecha CC, Sato TK, Nabet B, Hogenesch JB and Carstens RP: ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing. Mol Cell *33*: 591-601, 2009. PMID: 19285943. DOI: 10.1016/j.molcel.2009.01.025

31 Yamasaki L: Role of the RB tumor suppressor in cancer. Cancer Treat Res *115*: 209-239, 2003. PMID: 12613199. DOI: 10.1007/0-306-48158-8_9

32 La Starza R, Crescenzi B, Pierini V, Romoli S, Gorello P, Brandimarte L, Matteucci C, Kropp MG, Barba G, Martelli MF and Mecucci C: A common 93-kb duplicated DNA sequence at 1q21.2 in acute lymphoblastic leukemia and Burkitt lymphoma. Cancer Genet Cytogenet *175*: 73-76, 2007. PMID: 17498563. DOI: 10.1016/j.cancergencyto.2007.01.011

Received March 23, 2020
Revised April 6, 2020
Accepted April 7, 2020