Alternative splicing of DNA damage response genes and gastrointestinal cancers

Bahityar Rahmutulla, Kazuyuki Matsushita, Fumio Nomura

Bahityar Rahmutulla, Kazuyuki Matsushita, Fumio Nomura, Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan
Kazuyuki Matsushita, Fumio Nomura, Division of Laboratory Medicine, Chiba University Hospital, Chiba 260-8670, Japan

Author contributions: Rahmutulla B wrote the manuscript; Matsushita K edited the manuscript; and Nomura F supervised the manuscript.

Correspondence to: Kazuyuki Matsushita, MD, PhD, Associate Professor, Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. kmatsu@faculty.chiba-u.jp
Telephone: +81-43-2262167 Fax: +81-43-2262169
Received: June 11, 2014 Revised: July 18, 2014
Accepted: September 12, 2014
Published online: December 14, 2014

Abstract

Alternative splicing, which is a common phenomenon in mammalian genomes, is a fundamental process of gene regulation and contributes to great protein diversity. Alternative splicing events not only occur in the normal gene regulation process but are also closely related to certain diseases including cancer. In this review, we briefly demonstrate the concept of alternative splicing and DNA damage and describe the association of alternative splicing and cancer pathogenesis, focusing on the potential relationship of alternative splicing, DNA damage, and gastrointestinal cancers. We will also discuss whether alternative splicing leads to genetic instability, which is considered to be a driving force for tumorigenesis. Better understanding of the role and mechanism of alternative splicing in tumorigenesis may provide new directions for future cancer studies.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Alternative splicing; DNA damage; Gastrointestinal cancer; Mutation; Genetic instability

Core tip: Alternative splicing is a fundamental process of gene regulation in eukaryotes. Alternative splicing of DNA damage repair proteins is a significant cause of gene mutations, and those mutations in turn affect alternative splicing in cancer. Alternative splicing is associated with tumorigenesis by contributing to genetic instability. Therefore, alternative splicing of DNA damage response-related genes has an important role in tumorigenesis, survival, and growth of gastrointestinal cancers. In summary, the alternative splicing variants of these genes could be potential targets for both diagnosis and treatment of gastrointestinal cancers.

Rahmutulla B, Matsushita K, Nomura F. Alternative splicing of DNA damage response genes and gastrointestinal cancers. World J Gastroenterol 2014; 20(46): 17305-17313 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i46/17305.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i46.17305

INTRODUCTION

Alternative splicing is a fundamental process of gene regulation, which results in a single gene that codes for multiple proteins by excluding and/or including particular exons from pre-mRNA produced from that gene[1]. The process is performed by the spliceosome composed of five small nuclear ribonucleoproteins (snRNPs; U1, U2, U4, U5, and U6) and more than 100 different polypeptides[2]. In this process, many different types of proteins are translated from mRNA of the same gene origin and contribute to protein diversity. For example, at least 60% of human gene products undergo alternative splicing[3]. There are approximately 20000-35000 protein-
coding genes in a mammalian genome\(^5\), but the number of proteins generated by alternative splicing is much higher\(^6\) because many of these genes have multiple splicing patterns compensate up to thousands\(^7\). Thus, alternative splicing is a common phenomenon in the process of mammalian gene regulation and generation of protein diversity.

**DISTURBED ALTERNATIVE SPLICING IN HUMAN DISEASES**

Alternative splicing events may occur in both normal and disease-related gene regulation processes. The frequency of alternative splicing is higher in cancerous tissues than in normal tissues\(^8\). Occasionally, alternative splicing variants are expressed in cancer cells but not in normal cells. For example, far upstream element-binding protein (FBP)-interacting repressor (FIR) splice variants lacking or containing exon 2 and/or exon 5 are expressed in the majority of hepatocellular carcinomas (HCCs) but not in normal hepatocytes\(^9\). A well-known tumor suppressor gene p53 is alternatively spliced to produce at least twelve protein isoforms, which have important roles in cancer formation and progression\(^10\). It has been suggested that missense or silent mutations affect splicing\(^11\). According to the human gene mutation database, approximately 84% of hereditary diseases are associated with point mutations\(^12\). Teraoka et al\(^7\) suggested that 48% of these mutations result in defective splicing in the ATM gene in patients with ataxia-telangiectasia, and ATM has also been reported to be alternatively spliced in several types of cancer\(^13-15\). López-Bigas et al\(^16\) estimated that more than 60% of all disease-related mutations affect splicing. Lim et al\(^17\) suggested that 22% of disease alleles that were originally classified as missense mutations may also affect splicing and approximately one third of all disease-causing mutations alter pre-mRNA splicing. Alternative splicing variants of many genes and some well-known splicing factors have been reported to be associated with numerous cancers. For example, Ikaros family genes include Ikaros, Helios, and Aiolos. The Ikaros gene (ZNF384) is a member of the Kruppel transcription factor family characterized by the presence of zinc-finger domains located at their N- and C-termini and is alternatively spliced to give a number of variants\(^22\). Ikaros itself acts as a tumor suppressor in the lymphoid lineage\(^28\), but alternative splicing variants, such as Ik11, are aberrantly expressed in B-cell lymphoproliferative disorders and involved in tumor pathogenesis\(^29\). Helios was found to be abnormally spliced in adult T-cell leukemia, and deregulation of Helios expression promotes T-cell growth\(^23\). The splicing factor SRSF6 is an oncprotein reported to be over-expressed in lung and colon cancers\(^24\). Another splicing factor hnRNP has been suggested to be an oncogenic driver in glioblastoma\(^25,26\). Recurrent somatic mutations of splicing machinery genes, such as SF3B1, U2AF1, ZRSR2, and SRSF2, have been reported in numerous malignancies, including myelodysplastic syndromes, leukemias, and ovarian and gastric cancers\(^27-33\). Pre-mRNA processing factor 6 (PRPF6), a member of the tri-snRNP spliceosome complex, is required for alternative splicing of a number of genes, including ZAK kinase, and splicing activity of PRPF6 is important for colon cancer cell growth\(^34,35\). In addition to the associations shown in the above examples, many studies have suggested that alternative splicing is indeed closely related to certain diseases such as gastrointestinal cancers\(^36-48\).

**ABERRANT SPLICING OF DNA DAMAGE REPAIR GENES CAUSES GASTROINTESTINAL CANCERS**

Impaired DNA damage responses induce genetic instability. DNA double-stranded breaks represent one of the most severe types of DNA damage and promote genetic instability that is lethal to cells if left unrepaired\(^49,50\). Genetic instability includes two major categories: one is microsatellite instability, which involves subtle changes in DNA sequences (faulty DNA repair), and the other is chromosomal instability (CIN), which is characterized by gains and losses of whole or parts of chromosomes, and CIN is considered to be a driving force for tumorigenesis\(^51,52\). Single-stranded or double-stranded DNA breaks increase the susceptibility of chromosomal gross structural alterations that lead to CIN\(^51\). CIN is closely associated with the intrinsic multidrug resistance of cancer\(^53,54\). The possible association of DNA damage, alternative splicing, and genetic instability is schematically shown in Figure 1.

Chromosomal alterations are found in nearly all human cancers\(^55\). As mentioned above, severe types of DNA damage promote genetic instability and are an integral component of human neoplasia\(^56\). Alternative splicing affects the stability of transcripts by introducing premature STOP codons and directing mRNA degradation through the nonsense-mediated mRNA decay pathway\(^57\). Alternative splicing of DNA damage response genes promotes genetic instability. Therefore, alternative splicing is closely associated with DNA damage and tumorigenesis. Previous studies have shown that gastrointestinal cancers are closely associated with alternative splicing of DNA damage-related genes that cause genetic instability. For example, ATM is involved in the homologous recombination (HR) pathway of DNA repair, and MRE11 is a component of the DNA damage sensor MRN; these genes are found to be alternatively spliced in colon cancer cells\(^58,59\). Germline mutations in the DNA mismatch repair genes, MSH2, MLH1, MSH6 and PM2, are the cause of colon cancer, called Lynch syndrome\(^60,61\), and they are reported to be spliced in a number of gastrointestinal cancers\(^62-65\). Splicing factor 3b (SF3b) is a subcomplex of the U2 snRNP in the spliceosome\(^66\). SAP155 (a subunit of SF3b) is required for proper FIR expression and *vice versa*, and SAP155 knockdown or SF3b inhibition disrupts alternative splicing of FIR pre-mRNA and
generates FIRexon2[49]. FIR also acts as a molecular sensor for bleomycin-induced DNA damage by potentially interacting with DNA-PKcs and Ku-86/XRCC5[60] and has been reported to be alternatively spliced in colorectal cancer[40] as well as in HCCs[9]. Multifunctional splicing factor U2AF65, which has biotinylated triplex DNA affinity, has been reported to be associated with colorectal cancers[61]. Poly (ADP-ribose) polymerase (PARP)-1 is involved in single-stranded DNA damage repair and has a control role in the HR pathway[62]. PARP-1 is activated by Helicobacter pylori in the development and proliferation of gastric cancer[63]. The tumor suppressor genes, BRCA1 and BRCA2, are involved in DNA damage repair through their association with the HR mediator, RAD51, and their mutations are usually known to contribute to the tumorigenesis of hereditary breast and ovarian cancers[64]. Recent studies have further suggested that BRCA1 mutations in females below the age of 50 years increase the risk of colorectal cancer[65], and BRCA2 mutations are closely associated with pancreatic carcinogenesis[66,67]. RING finger protein 43, which is an E3-type ubiquitin ligase, has been reported to be mutated in pancreatic cancer[68] and gastric cancer[47] and was recently reported to act as a regulator of ATM-ATR DNA damage response; its mutation is associated with a high risk of developing sessile-serrated adenomas[48], which are believed to lead to colorectal cancer. The genes reported to have alternative splicing mutations in gastrointestinal cancers are summarized in Table 1. From the above examples, we can conclude that alternative splicing mutations in DNA damage response genes are closely associated with gastrointestinal carcinogenesis.

OTHER ALTERNATIVELY SPliced GENes THAT RELate TO GASTROINTESTINAL CANCERS

As mentioned above, alternative splicing is closely associated with gastrointestinal cancers and has an important role in their tumorigenesis. Gastrointestinal cancers are malignancies of the gastrointestinal tract and accessory organs of digestion, including the esophagus, stomach, biliary system, pancreas, small intestine, large intestine, rectum, and anus. They account for a large proportion of human malignancies and are a major cause of morbidity and mortality worldwide[68]. Among the gastrointestinal cancers, colorectal cancer is the third most frequently diagnosed cancer worldwide after lung and breast cancers, with 1.23 million diagnosed cases (9.7% of cancer diagnoses) in 2008[69]. There are many genetic and epigenetic changes that occur during colorectal carcinogenesis, including mutations of oncogenes, tumor suppressor genes, and mismatch repair genes; genetic instability; allelic losses in specific chromosomal arms; and methylation changes in gene promoters[70]. In addition, alternative splicing mutations have an important role in gastrointestinal carcinogenesis. In particular, alternatively spliced CD44 variants promote intestinal tumorigenesis induced by the activation of Wnt signaling[41]. Osteopontin splice variant (OPN-b) is found to be dominantly elevated in gastric cancer cell lines, and OPN-b has been shown to promote gastric cancer cell survival by regulation of Bel-2 family proteins and CD44v expressions[71]. The cyclin-dependent kinase inhibitor gene, which encodes P27, has
Table 1  List of alternatively spliced genes in gastrointestinal cancers

| Genes          | Role in DDR                                      | Gastrointestinal Cancers                  | Reference papers |
|----------------|--------------------------------------------------|--------------------------------------------|------------------|
| ATM, MSH2, MLH1, MSH6, PMS2 | DNA damage response kinase involved in HR pathway of DNA repair | Colorectal cancer and gastric cancers | [37-39,44,45,57] |
| MRE11          | Component of DNA damage sensor complex MRN       | Colorectal cancer                          | [36]             |
| PARP-1         | Involved in single stranded DNA damage repair    | Gastric cancer                            | [63]             |
| RNF43          | Plays role in controlling HR pathway             | Pancreatic cancer                         | [46]             |
| P53            | Activation by cellular stresses result in DNA damage inducer of EMT | Mediates EMT in colorectal cancer lines cancer | [84,85]         |
| P27 (CDKN1B)   | Cell cycle regulatory gene                       | Small intestine neuroendocrine tumors     | [72]             |
| Prrx1          | Paired related homoeobox 1, a newly reported EMT inducer | Gastrointestinal stromal tumors           | [75]             |
| HDM2           | Human double minute 2, negative regulator of p53 | Colorectal cancer                         | [79]             |
| PKM2           | Pyruvate kinase M2 gene, inactive state is associated with tumor cell proliferation | Impaired colorectal cancer growth         | [82]             |
| BRAF           | Raf kinase family member BRAF is a proto-oncogen replays a role in regulating the MAP kinase/ERKs signaling pathway | Malignant melanomas                       | [77]             |
| BMP            | Bone morphogenetic proteins, a group of growth factors, function in the formation of bone and cartilage, constitute morphogenetic signals etc. | Gastric cancer                           | [42]             |
| PRPF6          | Pre-mRNA processing factor 6, a member of the tri-snRNP spliceosome complex | Colon cancer                             | [34,35]          |
| Dystrophin     | Cause of Duchenne muscular dystrophy             | Metastatic GIST                           | [76]             |
| FGFR2          | The fibroblast growth factor receptor 2, encodes for a fibroblast growth factor-activated transmembrane receptor tyrosine kinase | Pancreatic ductal adenocarcinoma          | [91]             |
| Splicing factors in other cancers |                                      |                                            |                  |
| SRSF6          | Splicing factor                                   | Lung and colon cancers                    | [26]             |
| hnRNP          | Splicing factor                                   | Glioblastoma                              | [27,28]          |
| SF3B1, U2AF1   | Splicing factors                                  | Associated with numerous malignancies     | [29-33]          |
| ZRSR2, SRSF2   |                                                |                                            |                  |
| B11 (Ikars)    | Alternative splicing variant of Ikars, a member of Ikars family genes | B-cell lympho-proliferative disorders     | [24]             |
| Helas          | A member of Ikars family genes                    | T-cell leukemia                           | [25]             |
| FGF60 (FIR)    | FIR lacks exon5 of FGF60. FGF/FIR/F60 interacts with SF3B1 | Colon cancer, leukemia                     | [40,80,97]       |
| hnRNPM         | RNA-binding protein heterogeneous nuclear ribonucleoprotein M | Breast cancer metastasis                 | [92]             |

HR: Homologous recombination; DDR: DNA damage response; EMT: Epithelial-mesenchymal transition; GIST: Gastrointestinal stromal tumors.

been reported to have recurrent somatic mutations in small intestinal neuroendocrine tumors\(^{69}\). P27 was shown to be associated with proliferative activity of gastric cancer\(^{69}\). Approximately 85%-95% of gastrointestinal stromal tumors (GIST) have mutations in the \(c-KIT\) gene\(^{69}\). Dystrophin is expressed in the nonneoplastic and benign counterparts of GIST, but inactivation of dystrophin was observed in 96% of metastatic GIST. Deletion of the dystrophin-encoding and muscular dystrophy-associated DMD gene through alternative splicing led to inactivation of larger dystrophin isoforms and contributed to tumor formation and metastasis\(^{69}\). Mutations in the bone morphogenetic protein signaling pathway led to the development of juvenile polyposis syndrome, which increases the risk of gastric cancer development\(^{69}\). The Raf kinase family member, BRAF, is a proto-oncogene that has been reported to be frequently mutated in numerous human cancers, such as somatic missense mutations, in 66% of malignant melanomas and at lower frequency in colorectal cancers\(^{71}\). Murine double minute 2, which is a negative regulator of the tumor suppressor gene p53, was shown to be alternatively spliced under DNA dam-
Alternative splicing variants of certain genes not only have important roles in tumorigenesis but also significantly contribute to cancer metastasis. For example, the transcription factor, AP4, is encoded by the p53 tumor-suppressor gene and activated by numerous cellular stresses, which generally result in DNA damage[85]. AP4 is an inducer of epithelial-mesenchymal transition (EMT) and mediates c-MYC-induced EMT in colorectal cancer cell lines[86]. EMT of tumor cells contributes to metastasis[86,87]. Mesenchymal-epithelial transition (MET), which presumably contributes to tumor suppression[88], has been shown to be induced by p53 activation. Most recently, Peng et al[89] summarized the role of EMT in gastric cancer and suggested that loss of E-cadherin via its transcriptional repressors, such as Snail, ZEB, and Twist, is a key step in EMT activation, which significantly contributes to gastric carcinogenesis. Fibroblast growth factor receptor 2 (FGFR2) encodes for a fibroblast growth factor-activated transmembrane receptor tyrosine kinase and has been shown to be associated with EMT-related alternative splicing[90]; its alternative splicing generates the ΙΙb and ΙΙc isoforms. FGFR-2 ΙΙb expression correlates with venous invasion of pancreatic ductal adenocarcinoma, whereas FGFR-2 ΙΙc expression correlates with faster development of liver metastasis[91]. RNA-binding protein heterogeneous nuclear ribonucleoprotein M promotes breast cancer metastasis by activating the switch of alternative splicing that occurs during EMT[92].

Recently, splicing of paired related homeobox 1 (Prrx1) has been reported to be a novel EMT-MET switch. Alternative splicing of Prrx1 results in two variants, Prrx1a and Prrx1b, and the ratio of Prrx1a (with inhibition domain)/Prrx1b (lack of inhibition domain)[93] switches EMT-MET of cells and controls migration and invasion of pancreatic cancer[94]. Notably, Prrx1 is involved in metastasis and poor prognosis in colorectal cancer[95].

### CLINICAL APPLICATION OF ALTERNATIVE SPlicing TO CANCER

#### DIAGNOSIS AND TREATMENT

Alternative splicing variants can have potential targets for the diagnosis and treatment of many cancers, including gastrointestinal cancers (Figure 2)[96,97]. Novel splicing variants of FIR were generated by SAP155 siRNA, and these variants were also found to be activated in human colorectal cancer tissues[97]. Circulating FIR and FIRΔexon2 mRNAs are potential novel screening markers for colorectal cancer testing with conventional carcino-embryonic antigen and carbohydrate antigen 19-9. Given the central role of c-Myc in the development of many cancers, one direction toward the development of cancer gene therapies directed against c-Myc may go through FIR and its variants. The *Sendai virus* vector of FIR has shown strong tumor growth suppression with no significant side effects in an animal xenograft model and is potentially applicable to future clinical cancer treatment[98].

### CONCLUSION

Alternative splicing is a fundamental process of gene regulation in eukaryotes. It is a common phenomenon in mammalian genomes because most human genes undergo this process[9]. Alternative splicing leads to genetic instability, such as CIN, which drives tumorigenesis. DNA damage is one of the major reasons for genetic instabilities, and major components of the DNA damage repair pathway are alternatively spliced in certain cancers. Therefore, alternative splicing is closely associated with tumorigenesis by contributing to genetic instability. Alternative splicing of DNA damage repair proteins is a significant cause of gene mutations, which reciprocally affects alternative splicing in cancer. DNA damage promotes genetic instability, and genetic instability further promotes tumorigenesis (Figure 1). Genetic instability caused by certain types of DNA damage may be critical for the development of all colorectal cancers[95]. Many genes involved in the DNA damage repair pathway are alternatively spliced in gastrointestinal cancers (Table 1). Thus, the alternative splicing in DNA damage response-related genes has an important role in the tumorigenesis, survival, and growth of gastrointestinal cancers. Establishing a well-organized database of alternative splicing

[96,97]
would be helpful for facilitation of the process of considering a set of splice isoforms or their common regulatory network as targets of diagnostic or therapeutic strategies. Better understanding of the role and mechanism of alternative splicing in tumorigenesis may lead to novel directions for future cancer studies.

REFERENCES

1. Black DL. Mechanisms of alternative pre-messenger RNA splicing. Annu Rev Biochem 2003; 72: 291-336 [PMID: 12626338]
2. Wahl MC, Wall CL, Lührmann R. The spliceosome: design principles of a dynamic RNP machine. Cell 2009; 136: 701-718 [PMID: 19239890 DOI: 10.1016/j.cell.2009]
3. Modrek B, Lee C. A genomic view of alternative splicing. Nat Genet 2002; 30: 13-19 [PMID: 11753382]
4. Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat Genet 2008; 40: 1413-1415 [PMID: 18978789 DOI: 10.1038/ng.259]
5. Ewing B, Green P. Analysis of expressed sequence tags indicates 35,000 human genes. Nat Genet 2000; 25: 232-234 [PMID: 10835644]
6. Sultan M, Schulz MH, Richard H, Magen A, Klingenhoff A, Scherf M, Seifert M, Borodina T, Soldatov A, Parkhomchuk D, Schmidt D, O’Keeffe S, Haas S, Vingron M, Lehrach H, Yasp ML. A global view of gene activity and alternative splicing by deep sequencing of the human transcriptome. Science 2008; 321: 956-960 [PMID: 18599741 DOI: 10.1126/science.1160342]
7. Black DL. Protein diversity from alternative splicing: a challenge for bioinformatics and post-genome biology. Cell 2000; 103: 367-370 [PMID: 11081623]
8. Kim E, Goren A, Ast G. Insights into the connection between cancer and alternative splicing. Trends Genet 2008; 24: 7-10 [PMID: 18054115]
9. Malz M, Bovet M, Samarj, Rabenhorst U, Stichtenoth C, Bissinger M, Roessler S, Bermejo JL, Renner M, Calvisi DF, Singer S, Ganzinger M, Weber A, Gretz N, Zörnig M, Schirmacher P, Breuhahn K. Overexpression of far upstream element (FUSE) binding protein (FBP)-interacting repressor (FIR) supports growth of hepatocellular carcinoma. Hepatology 2014; 60: 1241-1250 [PMID: 24824848 DOI: 10.1002/hep.27218]
10. Surget S, Khoury MP, Bourdon JC. Uncovering the role of p53 splice variants in human malignancy: a clinical perspective. Onco Targets Ther 2013; 7: 57-68 [PMID: 24379683 DOI: 10.2147/OTT.S53876]
11. López-Bigas N, Audit B, Ouzounis C, Parra G, Guigó R. Are splicing mutations the most frequent cause of hereditary disease? FEBS Lett 2005; 579: 1900-1903 [PMID: 15792793]
12. Mazoyer S, Puget N, Perrin-Vidoz L, Lynch HT, Serova-Sinilnikova OM, Lenoir GM. A BRCA1 nonsense mutation causes exon skipping. Am J Hum Genet 1998; 62: 713-715 [PMID: 9497265]
13. Cartegni L, Chew SL, Kainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nat Rev Genet 2002; 3: 285-298 [PMID: 11967553]
14. Sanz DJ, Acedo A, Infante M, Durán M, Pérez-Cabosmero L, Esteban-Carderosa E, Lastra E, Pagani F, Patern C, Velasco E.A. A high proportion of DNA variants of BRCA1 and BRCA2 is associated with aberrant splicing in breast/ovarian cancer patients. Clin Cancer Res 2010; 16: 1957-1967 [PMID: 20215541 DOI: 10.1186/1078-0432.CCR-09-2564]
15. Rouleau E, Lefol C, Moncoutier V, Castella L, Houdayer C, Caputo S, Béche I, Buissen M, Mazoyer S, Stoppa-Lyonnet D, Noqué C, Lidereau R. A missense variant within BRCA1 exon 23 causing exon skipping. Cancer Genet Cytogenet 2018; 202: 144-146 [PMID: 20857879 DOI: 10.1016/j.cancergen.2010.07.122]
16. Stenson PD, Ball EV, Mott M, Phillips AD, Shiel JA, Thomas
Alternative splicing in gastrointestinal cancers

Rahmuitula B et al.

17 NS, Abesinghe S, Krawczak M, Cooper DN. Human Gene Mutation Database (HGMD): 2003 update. Hum Mutat 2003; 21: 577-581 [PMID: 12754702]

18 Terraoka SN, Telatar M, Becker-Catania S, Liang T, Onengut-Gumus, Toluay A, Chessa L, Sandal O, Bernatowska E, Gatti RA. Con-cannon P. Splicing defects in the axatia-telangiectasia gene, ATM: underlying mutations and consequences. Am J Hum Genet 1999; 64: 1617-1631 [PMID: 10330348]

19 Ejima Y, Yang L, Sasaki MS. Aberrant splicing of the ATM gene associated with shortening of the intron-monomerule-tide tract in human colon tumor cell lines: a novel mutation target of microsatellite instability. Int J Cancer 2000; 86: 262-268 [PMID: 10793255]

20 Ham MF, Takakawa T, Luo WJ, Liu A, Horii A, Aozasa K. Impairment of double-strand breaks repair and aberrant splicing of ATM and MRE11 in leukemia-lymphoma cell lines with microsatellite instability. Cancer Sci 2006; 97: 226-234 [PMID: 16542220]

21 Thorstensen YR, Roxas A, Kroiss R, Jenkins MA, Yu KM, Pearson T, Sengupta S, Boulos PB. The role of alternative-splicing: aberrant splicing promotes colorectal cancer. EMBO J 2013; 32: 630-639 [PMID: 23132731 DOI: 10.1002/pat.4129]

22 Winandy S, Wu P, Georgopoulos K. A dominant mutation in the Ikaros gene leads to rapid development of leukemia and lymphoma. Cell 1995; 83: 289-299 [PMID: 7585946]

23 Capece D, Zazzaroni F, Mancarelli MM, Verzella D, Fischi-metti M, Di Tommaso A, Maccaroni R, Plebani S, Di Ianni M, Gulino A, Alesse E. A novel, non-canonical splice variant of the Ikaros gene is aberrantly expressed in B-cell lymphoma. Leukemia 2013; 27: 979-987 [PMID: 23843578 DOI: 10.1038/leuk.2012.130]

24 Cohen-Elia M, Golan-Gerstl R, Siegfried Z, Andersen CL, Thorsen K, Ørntoft TF, Mu D, Karni R. The splicing factor SRSF6 is amplified and is an oncoprotein in lung and colorectal cancers. J Pathol 2013; 229: 630-639 [PMID: 23132731 DOI: 10.1002/path.4129]

25 Golan-Gerstl R, Cohen M, Shilo A, Suh SS, Bakacs A, C copola L, Karni R. Splicing factor hnRNPA2/B1 regulates tumor suppressor gene splicing and is an oncogenic driver in glioblastoma. Cancer Res 2011; 71: 4444-4452 [PMID: 21586613 DOI: 10.1158/0008-5472.CAN-10-4410]

26 Lefave CV, Squatrito M, Vorlova S, Rocco GL, Brennan CW, Haldorson EC, Pan YX, Cartegni L. Splicing factor hnRNPH2 drives an oncogenic splicing switch in gliomas. EMBO J 2011; 30: 4084-4097 [PMID: 21915099 DOI: 10.1038/emboj.2011.259]

27 Je EM, Yoo NJ, Kim YJ, Kim MS, Lee SH. Mutational analysis of splicing machinery genes SF3BI, U2AF1 and SRSF2 in myelodysplasia and other common tumors. Int J Cancer 2013; 133: 260-265 [PMID: 23280334 DOI: 10.1002/ijc.28011]

28 Visconte V, Makishima H, Maciejewski JP, Liu RV. Emerging roles of the splicing machinery in myelodysplastic syndromes and other hematological disorders. Leuke-
Rowan AJ, Gorman P, Halford S, Hustinx SR, Offerhaus GJ, Maitra A. Pancre...

Schultz N, Lopez E, Saleh-Gohari N, Helediday T. Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination. *Nucleic Acids Res* 2003; 31: 4959-4964 [PMID: 12909044]

Nossa CW, Jain P, Tamilselvam B, Gupta VR, Chen LF, Schreiber V, Desnoyers S, Blanke SR. Activation of the abundant nuclear factor poly(ADP-ribose) polymerase-1 by Helicobacter pylori. *Proc Natl Acad Sci USA* 2009; 106: 1998-20003 [PMID: 18997724 DOI: 10.1073/pnas.0906735108]

Woodford-Richens KL, Bastin GJ, Offerhaus GJ, Maitra A. Colorectal cancer in the presence of BRCA1 and BRCA2 mutation carriers: results from a follow-up study. *Br J Cancer* 2014; 110: 530-534 [PMID: 24292448 DOI: 10.1038/bjc.2013.741]

Kaur G, Saif MW. Translational research in pancreatic adenocarcinoma. *JOP* 2014; 15: 121-123 [PMID: 24618433 DOI: 10.6092/1590-8577/2327]

Jinsen RH, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; 127: 2893-2917 [PMID: 2135269]

Jass JR. Colorectal cancer: a multipathway disease. *Crit Rev Oncol Hematol* 2006; 62: 273-297 [PMID: 17425506]

Tang X, Li J, Yu B, Su L, Yu Y, Yan M, Liu B, Zhu Z. Osteopontin splice variants differentially exert clinicopathological features and biological functions in gastric cancer. *Int J Biol Sci* 2013; 9: 55-66 [PMID: 23289017 DOI: 10.7150/ijbs.5280]

Francis JM, Kiezun A, Ramos AH, Serra S, Pedamallu CS, Qian ZR, Banck MS, Kanwar R, Kulkarni AA, Karpathakis A, Manzo V, Contractor T, Phillips J, Nickerson E, Pho N, Hooshmand SM, Brais LK, Lawrence MS, Pugh T, McKenna A, Sivachenko A, Cibulskis K, Carter SL, Ainsworth P, Kim-Sing C, Meyn M, Ohh M. K63-ubiquitylation of VHL by SOCS1 mediates intrinsic multidrug resistance. *Mol Cancer* 2012; 11: 187 [PMID: 22731511 DOI: 10.1186/1476-4598-11-187]

Aoyagi K, Kouhui K, Miyagi M, Imazumi T, Kizaki J, Isobe T, Shirouzu K. Expression of p27Kip1 protein in gastric carcinoma. *Hepatogastroenterology* 2013; 60: 390-394 [PMID: 23858559]

Jee H, Lee SH, Park JW, Lee BR, Nam KT, Kim DY. Conexin32 inhibits gastric carcinogenesis through cell cycle arrest and altered expression of p21cip1 and p27Kip1. *BMB Rep* 2013; 46: 25-30 [PMID: 23351380]
85 \textbf{Davies} H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Haves R, Hughes J, Kosmicki V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Hakulinen B, Cooper C, Chipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenexv-Trench G, Riggins GJ, Bignier DD, Palmeri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Godwin AK, Pettitt RK, Stratton MR, Goodey ES, Manoukian VS, Sethi KD, de Rijn M, Ordog T, Kunkel LM, Fletcher JA. Dystrophin is associated with induced epithelial-mesenchymal transition in rat bladder carcinoma cells. \textit{Mol Cell Biol} 1994; 5: 851-862.

86 Matsuda Y, Yoshimura H, Suzuki T, Uchiida E, Naito Z, Ishiwata T. Inhibition of fibroblast growth factor receptor 2 attenuates proliferation and invasion of pancreatic cancer. \textit{Cancer Sci} 2014; 105: 1212-1219. DOI: 10.1111/cas.12470.

87 Xu Y, Gao XD, Lee JH, Huang H, Tan H, Akin J, Reineke LM, Peter ME, Feng Y, Gious D, Siziopikou KP, Peng J, Xiao X, Cheng C. Cell type-restricted activity of hnRNPM promotes breast cancer metastasis via regulating alternative splicing. \textit{Genes Dev} 2014; 28: 1191-1203. DOI: 10.1101/gad.241968.114.

88 Norris RA, Kern MJ. The identification of Prx1 transcription regulatory domains provides a mechanism for unequal compensation by the Prx1 and Prx2 loci. \textit{J Biol Chem} 2001; 276: 26829-26837. DOI: 10.1101/gad.1137327.

89 Reichert M, Takeo S, von Burstin J, Kim SB, Lee JS, Ihida-Stansbury K, Hahn C, Heeg S, Schneider G, Rhim AD, Stanger BZ, Rustgi AK. The Prx1 homeodomain transcription factor plays a central role in pancreatic regeneration and carcinogenesis. \textit{Genes Dev} 2013; 27: 288-300. DOI: 10.1101/gad.204453.112.

90 Takahashi Y, Sawada G, Kurashige J, Uchi R, Matsumura T, Ueo H, Takeo Y, Akiyoshi S, Eguchi H, Sudo T, Sugimachi K, Doki Y, Mori M, Mimori K. Paired related homoeobox 1, a new EMT inducer, is involved in metastasis and poor prognosis in colorectal cancer. \textit{Br J Cancer} 2013; 109: 307-311. DOI: 10.1038/bjc.2013.339.

91 Matsushita K, Tomonaga T, Kajiwara T, Shimada H, Itoga S, Hiwasa T, Kubo S, Ochiai T, Matsubara H, Nomura F. c-myc suppressor FBP-interacting repressor for cancer diagnosis and therapy. \textit{Front Biosci} (Landmark Ed) 2009; 14: 3401-3408. DOI: 10.2743/FBS.14.3401-3408.

92 Kajiwara T, Matsushita K, Itoga S, Tamura M, Tanaka N, Tomonaga T, Matsubara H, Shimada H, Habara Y, Matsuo M, Nomura F. SAP155-mediated c-myc suppressor far-upstream element-binding protein-interacting repressor splicing variants are activated in colon cancer tissues. \textit{Cancer Sci} 2013; 104: 149-156. DOI: 10.1111/cas.12058.

93 Matsushita K, Shimada H, Ueda Y, Inoue M, Hasegawa M, Tomonaka T, Matsubara H, Nomura F. Non-transmissible Sendai virus vector encoding c-myc suppressor FBP-interacting repressor for cancer therapy. \textit{World J Gastroenterol} 2014; 20: 4316-4328. DOI: 10.3748/wjg.v20.i15.4316.
