Splice isoforms as therapeutic targets for colorectal cancer

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Alternative pre-mRNA splicing allows exons of pre-mRNA to be spliced in different arrangements to produce functionally distinct mRNAs. More than 95% of human genes encode splice isoforms, some of which exert antagonistic functions. Recent studies revealed that alterations of the splicing machinery can cause the development of neoplasms, and understanding the splicing machinery is crucial for developing novel therapeutic strategies for malignancies. Colorectal cancer patients need novel strategies not only to enhance the efficacy of the currently available agents but also to utilize newly identified therapeutic targets. This review summarizes the current knowledge about the splice isoforms of VEGFA, UGT1A, PXR, cyclin D1, BIRC5 (survivin), DPD, K-RAS, SOX9, SLC39A14 and other genes, which may be possible therapeutic targets for colorectal cancer. Among them, the VEGFA splice isoforms are classified into VEGFαxx and VEGFαxxb, which have proangiogenic and antiangiogenic properties, respectively; UGT1A is alternatively spliced into UGT1A1 and other isoforms, which are regulated by pregnancy X receptor isoforms and undergo further splicing modifications. Recently, the splicing machinery has been extensively investigated and novel discoveries in this research field are being reported at a rapid pace. The information contained in this review also provides suggestions for how therapeutic strategies targeting alternative splicing can be further developed.

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the second most common cause of cancer mortality: ~608,000 deaths are attributed to this disease annually (1). As indicated in the National Comprehensive Cancer Network (NCCN) guidelines version 1.2013 (2), the anticancer agents used for CRC include 5-fluorouracil (5-FU), irinotecan hydrochloride (CPT-11), oxaliplatin and molecularly targeted agents such as bevacizumab, cetuximab and panitumumab. According to the guidelines, in the adjuvant setting for stage II or III patients with resectable CRC, regimens including 5-FU-based agents and leucovorin with or without oxaliplatin are recommended, and for stage IV patients with unresectable CRC, regimens including 5-FU-based agents and leucovorin with oxaliplatin or CPT-11 and the addition of bevacizumab, cetuximab or panitumumab are considered.

Abbreviations: 5-FU, 5-fluorouracil; 2′-O-Me, 2′-O-methyl; 2′-MOE, 2′-O-methoxymethyl; YSS, 3′ splice site; 5′SS, 5′ splice site; BIR, baculovirus IAP repeat; BIRC5, baculoviral IAP repeat-containing 5; CCND1, cyclin D1; CDK, cyclin-dependent kinase; CPT-11, irinotecan hydrochloride; CRC, colorectal cancer; CRND, colorectal neoplasia differentially expressed; DPD, dihydropyrimidine dehydrogenase; ESE, exonic splice enhancer; ESS, exonic splice silencer; hnRNP, heterogeneous nuclear ribonucleoprotein; IAP, inhibitor of apoptosis protein; ISE, intrinsic splice enhancer; ISS, intrinsic splice silencer; pre-mRNA, precursor messenger RNA; PXR, pregnane X receptor; sRNA, small interfering RNA; SR protein, serine/arginine-rich protein; UGT1A1, UDP glucuronosyltransferase 1A1; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

(2). Over the past two decades, there have been advances in the treatment of CRC; however, patients need novel strategies not only to enhance the efficacy of the above agents but also to utilize newly identified therapeutic targets.

Alternative precursor messenger RNA (pre-mRNA) splicing is the process by which the exons of pre-mRNA are spliced in different arrangements to produce structurally and functionally distinct mRNAs and proteins (Figure 1A) (3). After the completion of the Human Genome Project in 2004, alternative splicing has been recognized as one of the most important mechanisms that maintains genomic and functional diversity. It is well known that >95% of human genes encode splice isoforms (4), some of which exert antagonistic functions. A recent study revealed that alterations of the splicing machinery can cause the development of myeloid neoplasms (5), and understanding the splicing machinery is crucial for the development of novel therapeutic strategies for malignancies. A recent report revealed that a series of functionally associated splice isoforms are simultaneously expressed under a common regulatory network (6), which supports the notion that an entire set of splice isoforms or their common regulatory network should be considered as therapeutic targets, rather than focusing on a single gene as a target. In this review, we summarize the current knowledge about the potential of using splice isoforms as therapeutic targets, focusing on CRC, and discuss the future work that needs to be done to develop therapeutic strategies targeting these splice isoforms.

Alternative pre-mRNA splicing

The first studies on pre-mRNA splicing were published in 1977 (7,8). Two regulatory factors have been the focus of most investigations related to the splicing machinery: cis-elements and trans-elements (Figure 1A). Among the cis-elements, consensus splice sites such as the 5′ splice site (5′SS; also known as a splice donor site), the branch point motif, the poly-pyrimidine tract ([Y]n) and the 3′ splice site (3′SS; also known as a splice acceptor site) are essential for pre-mRNA splicing. Splice enhancers and silencers are also categorized into cis-elements, both of which are important for the recognition of the 5′SS and 3′SS sites. Depending on their localization within the genome, splice enhancers and silencers are subdivided into exonic splice enhancers (ESEs), intronic splice enhancers (ISEs), exonic splice silencers (ESSs) and intronic splice silencers (ISSs). Cis-elements are bound by trans-elements. Among the trans-elements, spliceosomes are multiprotein complexes comprising ~200 subunits. Among the subunits of spliceosomes, serine/arginine-rich proteins (SR proteins, SRp) predominantly bind to ESEs and ISEs; in contrast, heterogeneous nuclear ribonucleoproteins (hnRNPs) commonly bind to ESSs and ISSs. In many cases, hnRNPs block spliceosome assembly, resulting in exon skipping. Recently, tissue- or organ-specific SR proteins and hnRNPs have been extensively investigated (9,10). Figure 1B shows several patterns of alternative splicing in which splice isoforms are generated: (i) exon skipping in which an alternative exon is excluded or included, (ii) intron retention between constitutive exons, (iii) inclusion of one of the exons in a mutually exclusive manner, (iv) use of alternative 5′SSs, (v) alternative 3′SSs, (vi) alternative initiation sites and (vii) alternative polyadenylation sites.

The normal expression profile is indicated in the lower left panel, whereas the aberrant splicing that is observed in malignancies is shown in the lower right panel of Figure 1A, and can be subclassified into two categories: (i) aberrant splice isoforms as individual transcripts and (ii) an aberrant expression profile of splice isoforms as an entire set of transcripts; both of which occur at the germ cell or somatic cell level. Herein the word ‘change’ is used to encompass both ‘genetic polymorphism’ and ‘genetic alterations’. The former...
Fig. 1. (A) The regulation of alternative pre-mRNA splicing and its alteration in malignancies (adapted from ref. 3). Cis-elements and trans-elements are indicated with rectangles and ellipses, respectively. In the nucleotide sequences, Y denotes a pyrimidine (U or C) and R denotes a purine (G or A). ESE, exonic splice enhancer; ESS, exonic splice silencer; hnRNP, heterogeneous nuclear ribonucleoprotein; ISE, intronic splice enhancer; ISS, intronic splice silencer; snRNP, small nuclear ribonucleoprotein; SRp, serine/arginine-rich protein; SS, splice site; U2AF, U2 small nuclear ribonucleoprotein auxiliary factor. (B) Alternative pre-mRNA splicing (adapted from ref. 3). The green boxes indicate constitutive exons and the blue boxes indicate alternatively spliced exons.
category ‘aberrant splice isoforms’ can be caused by changes in the 5′-untranslated region (UTR), coding regions and 3′-UTR, as well as ESEs and ESSs, and altered expression of trans-elements may also cause aberrant splice isoforms. In contrast, the latter category ‘aberrant expression profiles’ can be caused by altered expression and structures of trans-elements, changes in the 5′-UTR, 3′-UTR, ESEs, ESSs, ISEs and ISSs, and possibly by changes of the consensus splice sites in introns. Recent evidence has demonstrated that most splicing occurs cotranscriptionally, and transcription modulates the splicing as well (11). In the following sections, the potential use of splice isoforms as therapeutic targets for CRC, all of which were recently identified, is discussed.

**Vascular endothelial growth factor**

The vascular endothelial growth factor (VEGF) gene superfamily consists of at least six ligands, many of which are spliced to generate a multitude of ligand isoforms (12). The VEGF molecules bind specifically to one or two of the three VEGF receptors (VEGFRs), with VEGF binding to VEGFR-1 and VEGFR-2 (13). VEGF and its receptors have been the most common research focus with regard to therapeutic targets with antiangiogenic effects. In addition to the previously known subfamily of VEGFA isoforms (VEGFAxxx), another subfamily, VEGFAxxxb, was identified in 2002 (14). The terminal exon B of VEGFA gene is spliced in a mutually exclusive manner, resulting in a six amino acid substitution (CDKPRR to SLTRKD) to generate VEGFAxxx and VEGFAxxxb, respectively (Figure 2A). Recent studies have revealed the VEGFAxxx isoform to have proangiogenic properties, whereas VEGFAxxxb has antiangiogenic properties (15,16).

Bevacizumab (17), which was approved for clinical use against CRC in 2004, is a humanized monoclonal antibody that inhibits both the VEGFAxxx and VEGFAxxxb isoforms by blocking their common kinase domain receptor binding site (18). Despite the effectiveness of bevacizumab when it is combined with cytotoxic agents, its low response rate, high rate of resistance and adverse events have been discussed (19). These disadvantages of using bevacizumab may be caused by non-specific targeting, probably resulting from the non-specificity of the antibody for the pro- and antiangiogenic isoforms. In response to these findings, strategies specifically blocking the VEGF/xxx subfamily have been explored and pegaptanib was developed as a short modified RNA aptamer that specifically binds to VEGF/xxx but not VEGF/xxxb (20). Another possible target for CRC is a trans-element, SRp55, which is known to increase VEGF/xxxb expression, leading to antiangiogenic effects (21). In addition, IGFI, TNF-α and TGF-β1 were also identified as being involved in regulating the alternative splicing of VEGFA (21).

**UDP glucuronosyltransferase 1A1 and pregnane X receptor**

CPT-11, a semisynthetic camptothecin derivative that functions as a topoisomerase I-inhibitor, has been used as an effective anticancer produg against CRC. CPT-11 is anabolized to its active metabolite, SN-38, by carboxylesterase (22), and catabolized to its inactive metabolite by glucuronidation (23). UDP glucuronosyltransferase 1A1 (UGT1A1) is the main enzyme involved in glucuronidation of UDP, and genetic polymorphisms of this enzyme, such as UGT1A1*28 (leading to a TA insertion in the promoter region) (24) and single nucleotide polymorphisms (25,26), are known to affect its glucuronidation activity. However, it is widely recognized that the UGT1A1 activity cannot be explained by the polymorphisms alone. One of the main reasons may be the alternative splicing of the UGT1A gene. Based on a search of the National Center for Biotechnology Information (NCBI) database and a PubMed literature survey, at least nine isoforms, including UGT1A1, are generated by the alternative splicing of UGT1A (Figure 2B), among which UGT1A1, UGT1A7, UGT1A9 and UGT1A10 have glucuronidation activity (27,28), but some of the other isoforms are non-functioning. This means that controlling the alternative splicing of UGT1A is important to avoid or decrease the adverse effects associated with CPT-11 and to enhance its efficacy. Recently, Guillemette’s group found that the UGT1A locus encodes a previously unknown splice isoform, UGT1A-i2, which is different from the previously known isoform, UGT1A-i1, which results from the alternative splicing of the terminal exon 5 (29). They found that UGT1A-i1 has glucuronosyltransferase activity, but UGT1A-i2 is inactive. By an immunohistochemical analysis using antibodies specific for each of the isoforms, they revealed that UGT1A-i1 and UGT1A-i2 are coproduced in the same structural regions in various organs (30). They further clarified that there is decreased expression of both UGT1A-i1 and UGT1A-i2 in CRC compared with corresponding normal tissues. Knockdown of endogenous UGT1A-i2 enhanced the cellular UGT1A-i1 activity (31), which supports the notion that UGT1A-i2 has a dominant-negative function and is a potential target for regulating the efficacy of CPT-11.

In addition, the UGT1A1 activity is regulated by splice isoforms of the pregnane X receptor (PXR) gene, which encodes a xenoreceptor that regulates drug metabolism and transporter genes (32). Currently, the PXR is known to have three major splice isoforms, T1, T2 and T3 (Figure 2C) (33). The expression of UGT1A1 isoforms, as well as that of UGT1A3 and UGT1A4, is upregulated by the T1 and T2 isoforms, but not by T3, which indicates that splice isoforms of PXR are potential therapeutic targets that may regulate the efficacy of CPT-11. In contrast, CPT-11 treatment of HCT116 cells preferentially affected the alternative splicing of factors such as RRMSA, which was not observed in cells treated with cisplatin or vinblastine (34). This indicates that the alternative splicing induced by CPT-11 was not simply due to reduced topoisomerase I activity, but rather was due to rapid RNA polymerase II hyperphosphorylation caused by CPT-11 (34).

**Cyclin D1**

The cyclin family is composed of proteins that control the progression of the cell cycle by activating cyclin-dependent kinases (CDKs). Among them, the protein encoded by CCND1 (cyclin D1) forms a complex with CDK4 and CDK6. The cyclin D1-CDK4/CDK6 complex induces the phosphorylation of retinoblastoma protein, which releases transcription factors from the phosphorylation of retinoblastoma protein complex, thereby promoting cell division through the G1-S checkpoint (35). For this reason, cyclin D1 has been regarded as a proto-oncogene and overexpression of cyclin D1 occurs at a high frequency in patients with CRC (36,37), esophageal cancer (38) and other malignancies. In addition, cyclin D1 can activate estrogen receptors in a CDK-independent manner in breast cancer (39) and an abundance of cyclin D1 affects the radiation sensitivity in some malignancies (40). The transcriptional mechanisms and other functions of cyclin D1 have recently been analyzed (41).

Although genetic alterations of the cyclin D1 locus are rarely observed, recent studies have demonstrated that the alternative splicing of cyclin D1 can influence the cancer risk and carcinogenesis (42). The cyclin D1 gene is known to produce two alternative splice isoforms: CD1a and CD1b (Figure 2D). CD1a is a canonical isoform that consists of five exons, whereas CD1b includes exons 1–4 and a partial intron 4 (43). In colon cancer and other malignancies, the single nucleotide polymorphism G870A, which is the last nucleotide of exon 4 (CCG and CCA) and is located adjacent to the 5′SS of intron 4 (GURAGU in Figure 1A), modulates the alternative splicing between exon 5 and intron 4, thus generating CD1a and CD1b, respectively (44). In addition, trans-elements ASF/SF2 (45) and Sam68 (46) regulate the alternative splicing toward the generation of CD1b. Although both CD1a and CD1b can associate with CDK4 and CDK6, they show distinct functions and cellular localizations. Phosphorylation of Thr286, which is located within exon 5 (Figure 2D), allows for the nucleocytoplasmic translocalization of cyclin D1 and its subsequent degradation (47); hence, CD1a can translocate to the cytoplasm, whereas CD1b remains constitutively in the nucleus. Although such functions of CD1a have not been observed, CD1b can cause cellular transformation and has been linked to human carcinogenesis (42,47). By performing the immunocytochemical analyses using antibodies for each of the isoforms, Li et al. (40) showed that CD1a, but not CD1b, elicited the DNA damage response in colon cancer cells when stably associated with chromatin. Considering the above results, the
Fig. 2. Splice isoforms of VEGFA (A), UGT1A (B), PXR (C), cyclin D1 (D) and survivin (E). For each of the genes, the pre-mRNA is indicated at the top and mature mRNAs are indicated below. White boxes indicate 5′-UTR and 3′-UTR. The NM numbers and the numbers of amino acids were provided based on the information contained in the NCBI database (as of 30 September 2012).
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two splice isoforms of cyclin D1 must be distinguished in order to develop therapeutic strategies targeting cyclin D1, and CD1b should be targeted for downregulation to maintain the inherent cell cycle control.

**Baculoviral inhibitor of apoptosis protein repeat-containing 5 (survivin)**

The inhibitor of apoptosis protein (IAP) family, which is characterized by the presence of baculovirus IAP repeat (BIR) domains (48,49), prevents apoptosis through direct inhibition of caspases and procaspases, and these proteins are expressed at elevated levels in the majority of human malignancies (50). Currently, seven genes in the IAP family have been isolated, among which the baculoviral IAP repeat-containing 5 (BIRC5) gene, also known as survivin, has been the most investigated as a therapeutic target for malignancies, and novel agents targeting this gene or protein are currently under development. Among them, YM155 (Astellas Pharma, Tsukuba, Japan) is a small molecule inhibitor of survivin (51). In 2012, Nakamura et al. (51) revealed that YM155 suppresses the expression of survivin through binding to the C-terminal region of interleukin enhancer-binding factor 3, although their study on the molecular mechanism is still underway. LY2181308 (Eli Lilly and Co., Indianapolis, IN) is a second-generation antisense oligonucleotide with a phosphorothioate backbone and other structural modifications, which targets the translation initiation site of the survivin transcripts (52). Both of these agents are designed to block all of the survivin transcripts. Recently, amiloride was reported to regulate the alternative splicing of survivin, as well as that of APAF1 and CRK (53).

Several splice isoforms of survivin have been reported (Figure 2E). In 2007, Sampath and Pelus (54) published a detailed review on the splice isoforms of survivin. The splice isoform Sur2B was regarded to be proapoptotic until the middle of the 2000s. However, the results of recent studies in CRC (55) and other malignancies (56,57) indicated different outcomes. In 2010, Sawai et al. (55) reported that Sur2B expression in CRC is an important factor involved in the invasive capacity of tumors in the presence of 5-FU. In 2011, Huang et al. (56) reported that the SurWT, Sur-DeltaEx3 and Sur2B isoforms were significantly elevated in astrocytoma and were associated with a poorer prognosis and Vivas-Mejia’s study on ovarian cancer cells showed that Sur2B was more abundant in taxane-resistant cells than in taxane-sensitive cells (57). Using CRC samples and corresponding normal tissues, Pavlidou et al. (58) analyzed the expression levels of the isoforms, and Antonacopoulou et al. (59) analyzed the correlation between the expression of survivin isoforms and single nucleotide polymorphisms. It is still difficult to integrate all of the information on the survivin isoforms because some of the results have been contradictory, but the information will be important to design therapeutic strategies targeting survivin.

**Dihydropyrimidine dehydrogenase**

After its development in 1957 (60), 5-FU has been a core anticancer agent used for CRC. Approximately 90% of the administered 5-FU is eliminated in the liver, whereas the remaining 10% of 5-FU is anabolized to exert cytotoxic activity (61), making DPD the most important determinant of toxicity. Several splice isoforms of survivin. The splice isoform Sur2B was regarded to be proapoptotic until the middle of the 2000s. However, the results of recent studies in CRC (55) and other malignancies (56,57) indicated different outcomes. In 2010, Sawai et al. (55) reported that Sur2B expression in CRC is an important factor involved in the invasive capacity of tumors in the presence of 5-FU. In 2011, Huang et al. (56) reported that the SurWT, Sur-DeltaEx3 and Sur2B isoforms were significantly elevated in astrocytoma and were associated with a poorer prognosis and Vivas-Mejia’s study on ovarian cancer cells showed that Sur2B was more abundant in taxane-resistant cells than in taxane-sensitive cells (57). Using CRC samples and corresponding normal tissues, Pavlidou et al. (58) analyzed the expression levels of the isoforms, and Antonacopoulou et al. (59) analyzed the correlation between the expression of survivin isoforms and single nucleotide polymorphisms. It is still difficult to integrate all of the information on the survivin isoforms because some of the results have been contradictory, but the information will be important to design therapeutic strategies targeting survivin.

**Therapeutic strategies to target splice isoforms**

Therapeutic targeting of splice isoforms may be achieved through conventional small molecules, but these molecules can only target a small subset of proteins, such as enzymes (e.g. tyrosine kinases) and receptors (e.g. the epidermal growth factor receptor). On the other hand, RNA-based therapeutics can theoretically target all of the pre-mRNAs and mRNAs with a wider range and higher selectivity than small molecules (80), although almost all of these modalities are still in preclinical development. Currently, the most important issue to be resolved for the use of RNA-based therapeutics as macromolecules is the development of an optimal drug delivery system.

The RNA-based therapeutics include antisense oligonucleotides, small interfering RNA (siRNA), splice-switching oligonucleotides and other molecules such as ribozymes and aptamers. Among them, synthetically modified antisense oligonucleotides are about 20 nucleotides long (Figure 3A) and the annealing of the oligonucleotides...
Fig. 3. Macromolecules targeting splice isoforms associated with malignancy. (A) A synthetically modified antisense oligonucleotide, (B) siRNA, (C) a splice-switching oligonucleotide and (D) an antibody. In (C), an example of splice switching is presented, in which an ESE located in the aberrant exon is annealed to an oligonucleotide, and the aberrant exon is skipped. AGO, argonaute; ESE, exonic splice enhancer; RISC, RNA-induced silencing complex; RNase H, ribonuclease H.
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...to mRNA allows the cleavage of the mRNA by ribonuclease H. To provide enhanced structural stability and pharmacological qualities while not interfering with the activity of ribonuclease H, various modifications of their chemical structures have been made, such as the use of a phosphorothioate linkage instead of natural phosphates as a backbone of nucleotides, and 2′-O-methyl (2′-OMe) residues, 2′-O-methoxyethyl (2′-MOE) residues or locked nucleic acids (81) have also been developed. In addition, the chemistry-dependent toxicities induced by their structures is another important issue to be considered. siRNA (Figure 3B) is another modality that consists of a double-stranded RNA fragment 21–22 nucleotides long. After interacting with the multiprotein RNA-induced silencing complex, the antisense strand of the siRNA anneals to the complementary mRNA as a target, and the endonuclease argonaut 2 cleaves the annealed mRNA. In this modality, off-target effects and the innate immune response via the activation of Toll-like receptors should be carefully managed. Splice-switching oligonucleotides (Figure 3C) modulate pre-mRNA splicing with spliceosomes and repair the defective pre-mRNA to generate proteins that have distinct functions. Monoclonal antibodies (82) have also been used for various targets (Figure 3D). In contrast with the RNA-based therapeutics, some antibodies targeting oncogenic proteins have already been in clinical use; and those for each of the splice isoforms will likely be further developed for clinical use. The development of antibodies is still very expensive, and further considerations for their development are discussed elsewhere (83). Targeting trans-elements that act as spliceosomes or splicing modulators is another option.

Future perspectives

In this review, we summarized the splice isoforms that represent possible therapeutic targets for CRC. As discussed in this review, isoform-specific antibodies for VEGFA (16), UGT1A (30), cyclin D1 (40), K-RAS (64) and SOX9 (67) are currently available, and they can be utilized for immunohistochemical analyses and other purposes, and may eventually be useful for clinical applications. With the recent advances in nucleotide sequencing technologies, an entire set of genomic DNA sequences has been analyzed, and in the next stage, an entire set of RNA sequences will be further analyzed; the interpretation of the latter, however, is far more complex compared with the former, mainly due to the wide variety of mature mRNAs resulting from alternative splicing. To elucidate the regulatory mechanism(s) for alternative splicing as a whole, the two sets of sequence information will have to be integrated. Although the importance of individual cis-elements in the splicing machinery has been widely discussed, the concept of a ‘splicing code’, which is defined as a complex combination of the cis-elements that direct constitutive or alternative splicing, was proposed as early as the 1970s. To experimentally prove this concept had been a major challenge, but recent studies combining transcriptome-wide data with advanced machine learning algorithms were able to predict new classes of alternative splicing events under regulation by the splicing code (84,85). Furthermore, in a recent genome-wide siRNA screening, Moore et al. (6) identified a co-ordinated alternative splicing of Bcl-X, MCL1, CASP9 and other apoptosis-associated genes under a common regulatory network. These findings suggest that we should consider a set of splice isoforms or their common regulatory network when developing therapeutic strategies for malignancies, rather than targeting a single gene. To what extent the mechanisms regulating alternative splicing are organ-specific remains unclear, but the phenomenon is complex, and is the subject of many ongoing studies.

Research in these various areas is still ongoing, and new discoveries are being reported at a rapid pace. Recent reports have demonstrated that alternative splicing is also affected by newly identified regulatory factors, such as RNA polymerase II elongation (86), the chromatin structure (87,88), histone modifications (89), the RNA structure (90) and the spliceosome structure (91), most of which are interwoven bidirectionally (87,88). Importantly, the splicing machinery is regulated by innate microRNAs, siRNAs, small nucleolar RNAs and other non-coding RNAs (92,93), and these should also be considered as therapeutic targets. The rapidly increasing information available about nucleotide sequences, trans-elements, and newly identified regulatory factors, along with novel bioinformatics technology, such as the multi-mapping Bayesian gene eXpression (MMGX) program by Turro et al. (94), which enables the detection of differential splicing at the isoform level, will provide additional information about how therapeutic strategies targeting alternative splicing in malignancies can be developed.

Funding

HIROMI Medical Research Foundation (Sendai, Japan); Osaka Basic Medical Research Promoting Foundation (Osaka, Japan).

Conflict of Interest Statement: None declared.

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Received August 2, 2012; revised October 10, 2012; accepted October 25, 2012.