Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 334 (2005) 23–29
BBRC
www.elsevier.com/locate/ybbrc

Candidates for tumor-specific alternative splicing

Masayo Okumura [a,*], Shinichi Kondo [b], Maiko Ogata [a,b], Soshi Kanemoto [a,b], Tomohiko Murakami [a,b], Kanta Yanagida [b], Atsushi Saito [a,b], Kazunori Imaizumi [b,*]

[a] *Division of Structural Cellular Biology, The Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma, Nara 630-0101, Japan*

[b] *Division of Molecular and Cellular Biology, Department of Anatomy, Faculty of Medicine, University of Miyazaki, Kihara 5200, Kiyotake, Miyazaki 889-1692, Japan*

Received 7 June 2005
Available online 21 June 2005

Abstract

Gene expression can be regulated not only by transcription and post-transcriptional modifications, but also by splicing regulation. Recent genome-wide analyses have indicated that up to 70% of human genes may have alternatively spliced forms, suggesting that splicing regulation affects a wide range of gene expression. Tumor tissues show significantly altered protein expressions, and this is also thought to be affected by alternative splicing. Although some alternative splicing events have been reported to be cancer specific and others have been predicted from database analyses, the process of alternative splicing and its regulatory machinery are hardly understood. We searched for and detected alternative splicing events that alter protein splicing in all or a subset of tumor tissues. The results revealed tissue-specific alterations of splicing regulation by tumorigenesis, and regulatory *cis*-element analyses further suggested that multiple splicing regulatory machineries were affected by this process.

Keywords: Alternative splicing; Cancer; Tumorigenesis; Proteomics

Pre-mRNA splicing is a ubiquitous nuclear process, in which introns are excised and exons are ligated. Alternative splicing, which is now commonly thought to affect more than half of all human gene expression, generates the functional complexity of the proteome by producing several kinds of mRNA from one gene [1,2]. In addition, dysregulation of the splicing machinery, which causes misrecognition of 3′- or 5′-splice sites, generates aberrant mRNAs, and produces greater heterogeneity of pre-mRNA processing [3].

Many researchers have analyzed and detected cancer-specific protein expression patterns at the transcription or protein expression levels, and reported these as cancer markers [4]. However, protein expression can be regulated not only by transcription, translation or post-translational modifications, but also by the splicing regulation [5,6]. By changing the exon selection, the amino acid sequence of the protein encoded by the mRNA can be dramatically changed, or a stop codon created by a frameshift can destroy the open reading frame. Furthermore, although exons altered in the 3′- or 5′-untranslated region do not affect the amino acid sequence, they can influence the stability, transport efficiency or translational efficiency of mature mRNA, and also modify the protein expression.

In addition to many kinds of tissue-specific alternative splicing, it has been reported that some genes change their alternative splicing pattern in response to signals from the extracellular matrix [7–9], environmental stress [10,11] or tumorigenesis [12–17]. Such examples indicate that protein expression can be affected by

* Corresponding authors. Fax: +81 743 72 5419 (M. Okumura), +81 985 85 9851 (K. Imaizumi).
E-mail addresses: masayo@bs.naist.jp (M. Okumura), imaizumi@med.miyazaki-u.ac.jp (K. Imaizumi).

0006-291X/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2005.06.057

splicing regulation, and suggest that some cancer-specific protein expression patterns are caused by cancer-specific alternative splicing. Some alternative splicing events have been reported to be cancer specific from comparisons of normal tissues with tumor samples or tumor derived cell lines [12–16], while others have been based on analyses of databases, such as GenBank [18–22].

Here, we tried to detect cancer-specific alternative splicing and examined the regulatory mechanism. Using a splicing database established by comparing the UniGene clusters of human expressed sequence tags (ESTs) and the human genome sequence [22], as well as many other reports [12–17,23,24], we selected targets for our analyses and checked their cancer specificities. Furthermore, the detected cancer-specific examples were also examined for their regulatory mechanisms. The results strongly suggest the existence of cancer-specific alternative splicing and tissue-specific alterations, and indicate that a variety of mechanisms are influenced by tumorigenesis.

Materials and methods

Cell culture. The human colon carcinoma cell line SW480, human lung adenocarcinoma cell line A549, and human kidney renal clear cell carcinoma cell line Caki-1 were provided by the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer (Tohoku University, Japan), and maintained in RPMI1640 medium supplemented with 10% fetal calf serum. The human endometrial adenocarcinoma cell line Hec-1 was provided by the Health Science Research Resources Bank (Tokyo, Japan), and maintained in Eagle's minimum essential medium supplemented with 10% fetal calf serum.

RNAs and RT-PCR. Human total RNAs from normal and tumor tissues were purchased from Novagen. Other RNAs were prepared from tissues or cells using an RNeasy Mini Kit (Qiagen). A random hexamer primer and MMLV reverse transcriptase (Invitrogen) were used for reverse transcription according to the manufacturer's protocol. PCR was performed with r*Taq* DNA polymerase (TaKaRa) as follows: 1 min at 94 °C for denaturation, 30 cycles of 1 min at 94 °C, 30 s at 55 °C and 1 min at 72 °C for amplification, and 5 min at 72 °C for extension. The primers used for PCR were either gene specific and designed to detect endogenous splicing (listed in Table 1), or SA2 (ATCTCAGTGGTATTTGTGAGC) and SD6 (TCTGAGTCA CCTGGACAACC) to detect exon trapping products. The PCR products were electrophoresed in 7.5% acrylamide gels.

Exon trapping. Genome sequences around each target exon were amplified from human genomic DNA prepared from cultured cells using platinum *Taq Pfu* (Invitrogen), checked for their sequences, and then cloned into the *Bam*HI/*Eco*RI sites of the pSPL3 exon-trapping vector (Life Technologies), which was modified to be driven by the CMV promoter. The primers for amplification of the genomic fragments were as follows: et-TPM1: forward, GAATTCAGCATGACCTTCTGG CAGCT and reverse, GGATCCATATCAAATGGCACACTGCA; et-TPM1L: forward, GAATTCATGTGTATTTTATCCCTCCT and reverse, GGATCCTAAAATGGATCAGAAACTAC; et-ACTN1: forward, GAATTCGAGGGCACCCCCTGGAGCCT and reverse, GGATCCACCAGTCCTTCCTGCCAGCC; et-ITGB4: forward, GAATTCTTCCTCTCTTCCAGCTCCTG and reverse, GGATCCG CAGGCCCTGCTTGTGCCAA. The other vectors for exon-trapping analyses were constructed by modifying these.

Plasmids were transfected into cells using LipofectAMINE 2000 (Gibco) according to the manufacturer's protocol. At 24 h after transfection, total RNAs were prepared for RT-PCR.

Sequence analysis. The ASAP database (http://www.bioinformatics.ucla.edu/HASDB/) was used to search for cancer predominant alternative splicing. The primers for RT-PCR analysis of each target are described in Table 1. For some genes, a forward primer and a reverse primer could detect both of the alternatively spliced products, while other genes required an additional primer to be designed.

The sequence data were referred to the GenBank as follows; AL050179, tropomyosin 1 (α) (TPM1); NM_000213, integrin β 4 (ITGB4); NM_001102, actinin, α 1 (ACTN1); NM_018297, *N*-glycanase 1 (NGLY1); AF062341, catenin δ 1 (p120ctn); NM_022974, fibroblast growth factor receptor 2 (FGFR2); NM_001156, annexin A7 (ANXA7); NM_006112, peptidylprolyl isomerase E (PPIE).

Results

Detection of cancer-specific splicing

Lee et al. [22] have built up an online database termed as ASAP (Alternative Splicing Annotation Project), in which cancer predominant alternative splicing is listed with a predominancy score estimated from the UniGene EST database and the human genome sequence. However, it remains unknown whether the alternative splicing events listed as cancer specific actually alter the splicing patterns in tumor tissues. We selected 50 alternative splicing examples from the ASAP list as targets for our cancer-specificity analysis, based on their cancer predominancy score and the simplicity of their alternative splicing patterns for further analyses. In addition, nine targets were selected from alternative splicing examples reported to be cancer specific [12–17,25–27], and four targets from reported exclusive patterns of alternative splicing [23,24]. For each of these total 63 alternative splicing targets, specific forward and reverse primers were designed to detect both alternatively spliced products, and their endogenous splicing patterns in human normal and tumor tissues were compared. The examined tissues were the colon, kidney, lung, and uterus.

For 31 of the 63 examples, both the expected alternatively spliced products were detected. Among these 31 examples, 21 showed apparent differences between the normal and tumor alternative splicing patterns in at least one tissue, with six showing a difference in all four tumor tissues examined (Table 1). Fig. 1 shows the results for three of these six examples. *TPM1* showed the exclusive alternative splicing at exon 6. RT-PCR detected both the splicing products, which had to be distinguished by digestion with the restriction enzyme *Pvu*II since the lengths of the two alternative exons were the same. In all four tissues, the splicing event including exon 6a was more predominant in tumor tissues than in normal tissues. Interestingly, when four cell lines derived from tumor tissues corresponding to the four tissues

Table 1
Alternative splicing events examined in this study

Gene	UniGene No.	Normal/tumor				Forward-1	Forward-2	Reverse-1	Reverse-2
		C	K	L	U				
TPM1	Hs.133892	X	X	X	X	ACATTGCTGAAGATGCCGAC		TCAGCTTGTCGGAAAGGACC	
ITGB4	Hs.370255	X	X	X	X	TAAGCACATCCTCCACCCTC		ACTCTGAGAGATGTGGGCCC	
GAJ	Hs.294088	X	X	X	X	GGTTGACTGTGAGAGGATCG		ATTTCATGGAAGCAGCAGCC	AAGCTCTTTTGC TAGCCTGG
CPT1B	Hs.439777	X	X	X	X	CGTCATCATGGCAACAGTGG		ATGGCCATGCTGAGAAGTGC	
ACTN4	Hs.270291	X	X	X	X	CCGTATAAGAACGTCAATGTGC		GTTCTCTTGGTTGACAGCCAGC	
ACTN1	Hs.509765	X	X	X	X	GAGCAGATGAATGAGTTCCG		TGAAGTCAATGAAGGCCTGG	
CYP2C8	Hs.282871	X	X	—	—	TTTCACTCTGGAGACAGAGC		ATACACGGTGAACACAGGAC	
CYP2C8	Hs.282871	—	X	—	—	TTTCTCCCTCACAAACTTGC		GGGAGTTCAGAATCCTGAAG	
NGLY1	Hs.368960	—	—	—	X	TTTCTGGATGGGAGAATGGC		GACATCACCATCTCCTCTGC	
AKAP1	Hs.463506	X	—	—	—	ACCCCGATGGTAGCCGAGGA	CCTTGGTGGCAGAACCGAGC	CACCTGCTGCTCATCATGGC	
HRLP5	Hs.410316	—	X	—	—	CAGTACAGCCTGATTGAAGG		AACTCTTCCTCTAGCTGGAG	
WRB	Hs.198308	—	—	X	—	GTGCTCAGCTTCGTGTTTGG	GAGAGTTGTCCCTACTGTGC	AGAGCTCCTGCTTCATGTCC	
WDR4	Hs.248815	X	X	X	—	ATTCTGGCGTCCACCTTCTC		AGACGTCTCCAGACTTGTCG	
BAG5	Hs.5443	—	X	—	X	AGCTGCGGAAGTCGTGGAAG	CCGTAAAGGGCTGATCTTCC	CACTGAAGCCGATAACTTGC	
ZNF265	Hs.194718	—	X	—	—	CATCATCTTCTCCTGAGAGG		TTTGAACTTGAACGGGAACC	
MOXD1	Hs.6909	—	X	—	X	GAGGCTAAGGAGAATGGAAC		ACTGGGGTTTAGGTAGTGAG	TGATGATTCCTT TGAGGATG
CD44	Hs.502328	—	X	—	X	CCTACTGATGATGACGTGAGCAGCG		TCAGATCCATGAGTGGTATG GGACC	
p120ctn	Hs.166011	—	X	X	X	GAAGCCAGAGCAGTCATTCA		CCCAGATGGAACGGAGATA	
FLT4	Hs.415048	X	—	—	—	GCAGGCATAGACAAGAAAGC		CTTGTCCACTTTCATGCTCC	CGCTGTTGTAA AACACCTGG
KRT8	Hs.533782	—	—	—	X	CTATATGAAGAGGAGATCCG		TACATGCTCTCAGCCTCAGC	
FGFR2	Hs.533683	—	X	X	X	AGAACGGCAGTAAATACGGG		GCTATCTCCAGGTAGTCTGG	
BNIP1	Hs.145726	—	—	—	—	TGTTCAGGACCCTTAAGTGC		TGTGATTCTCCACTTCCTGG	
CREB3L1	Hs.405961	—	—	—	—	TGAACTGTGGAAGAAGGTGG		CTTTGGGCCAGAAAGGAACC	CACCAGAACAA AGCACAAGG
KCNAB2	Hs.440497	—	—	—	—	GTATCCAGAATCAACGACGG		ATGTTCCAAGTCCCAGGCAG	
ANXA7	Hs.386434	—	—	—	—	TTTCCTGGAGGACAGATGCC		ATAGCATCGAAGTTGGCAGC	
CCNDBP1	Hs.36794	—	—	—	—	TGTGAACAAGTCCATGCTGC		TTGGTATCTGAGGCATCTGC	
BCAS1	Hs.400556	—	—	—	—	GGTTTCACCTAACAAAGCTG		GCATCCTTTGGAATTCTTGC	
PPIE	Hs.524690	—	—	—	—	CTGGGAAGACGCTTGAAGAG		AAATTCTCTGCTGTCATGGG	
MCM7	Hs.438720	—	—	—	—	GATGGCACTGAAGGACTACG	ACGGGCTCTTTATTCTTCGC	GGTTCCCATACTTGAACTGC	
SERF2	Hs.424126	—	—	—	—	GCAACGTCCGACAGAACGAG		GCAGAAAGCCCGTCATCTCG	
ITGA6	Hs.133397	—	—	—	—	GAACTGTGTGAACATCAGA		TTCCCTCATACTTCGGATTGA	

Genes for which both the alternatively spliced products were detected are listed with their specific primer sequences. The splicing events in normal and tumor tissues are compared. C, colon; K, kidney; L, lung; U, uterus; X, a difference is detected; —, no difference is detected.

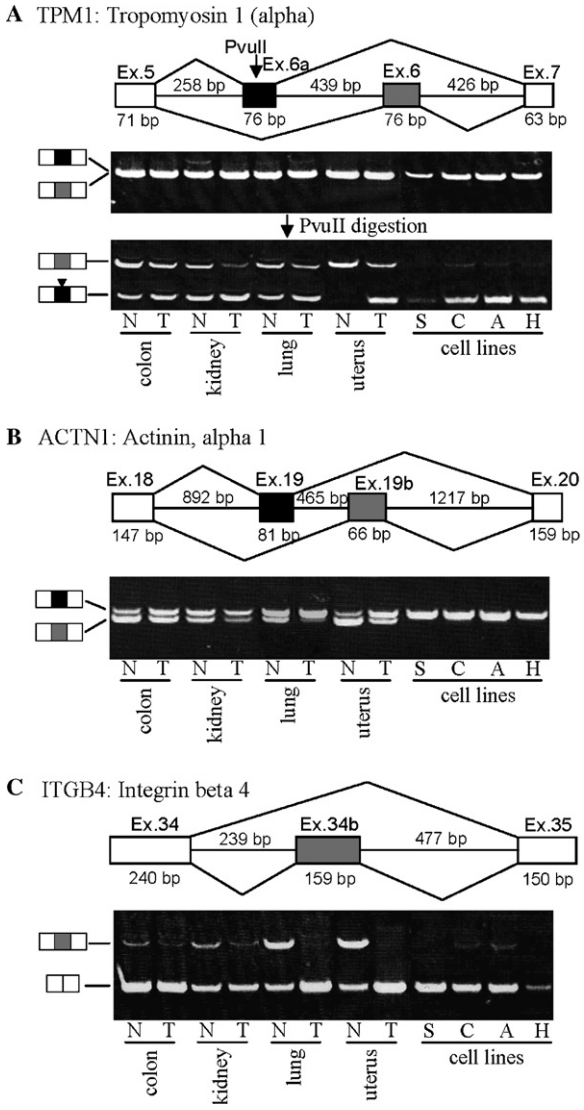

Fig. 1. Alternative splicing events influenced in all four tumor tissues. For each indicated gene, the genomic structure around the alternatively spliced exons with the sizes of the exons and introns are shown above. The transcripts in the indicated tissues or cells detected by RT-PCR are shown with their exon compositions below. The alternatively spliced exons are represented by black or gray boxes. Two fragments from *TPM1* were distinguished by *Pvu*II digestion. N, normal tissue; T, tumor tissue; S, SW480 cell line; C, Caki-1 cell line; A, A549 cell line; H, Hec-1 cell line.

were examined, the tumor splicing patterns were remarkable, i.e., almost all the splicing products in these cells contained exon 6a, rather than exon 6. *ACTN1* showed exclusive alternative splicing at exon 19. The forward exon (exon 19) was predominant in tumor tissues, as well as in the cell lines. *ITGB4* showed one alternatively spliced exon between exons 34 and 35. Suppression of the alternative exon (exon 34b) was more prominent in all four tumor tissues, as well as in the cell lines. Thus, the tumor predominant alternative splicing of these three examples was also predominant, or more remarkable, in the tumor derived cell lines, indicating that the alterations of the splicing machineries regulating these splicing events were maintained or enhanced in the cell lines.

Examples of genes that changed their alternative splicing patterns in only a subset of the tumor tissues are shown in Fig. 2. Inclusion of exon 10 of *NGLY1* was suppressed in tumors of the lung and uterus, while no difference was detected in tumors of the colon and kidney. The spliced product of *p120ctn* containing exons

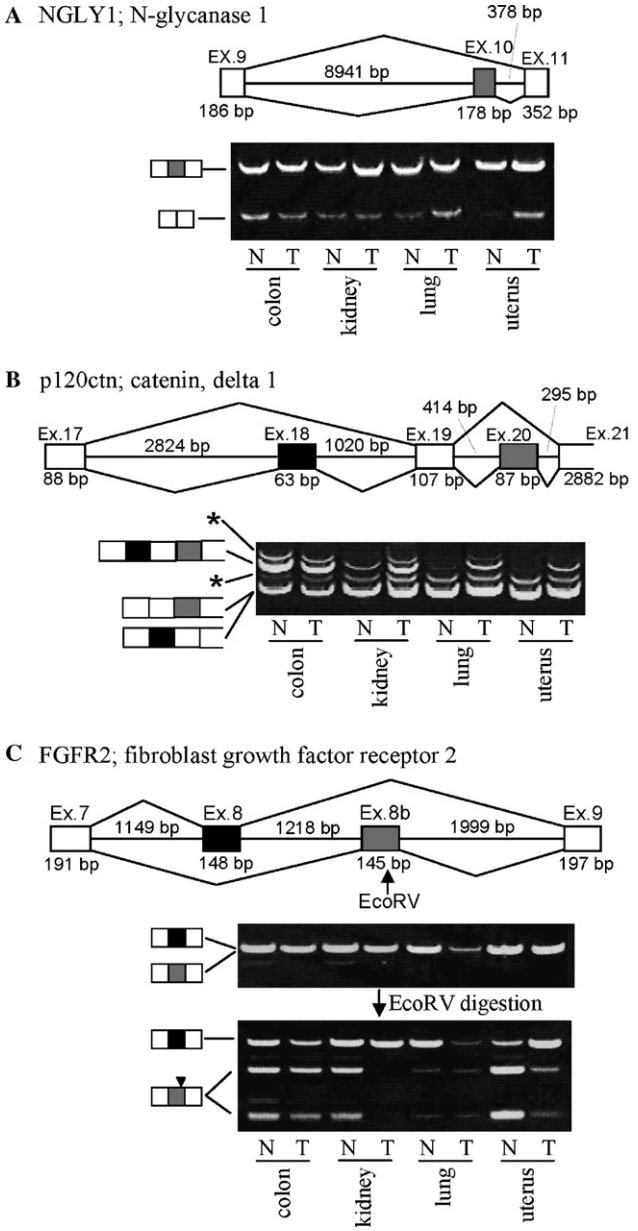

Fig. 2. Alternative splicing events influenced in a subset of tumor tissues. For each indicated gene, genomic structure around the alternatively spliced exons with the sizes of the exons and introns are shown above. The transcripts in the indicated tissues detected by RT-PCR are shown with their exon compositions below. The alternatively spliced exons are represented by black or gray boxes. Two fragments from *FGFR2* were distinguished by *Eco*RV digestion. N, normal tissue; T, tumor tissue; asterisks, unidentified fragments.

18, 19, and 20 was more abundant in tumors of the kidney, lung, and uterus, but not in tumor of the colon. The forward exon of the exclusive alternatively spliced exons in *FGFR2* was predominant in tumor of the kidney and uterus, similar to *TPM1* and *ACTN1*, whereas no change was observed in tumor of the colon and it was predominant in normal lung tissue rather than in lung tumor tissue.

Fig. 3 shows the alternative splicing patterns of *ANXA7* and *PPIE*, for which no alterations due to tumorigenesis were detected in any of the four tissues examined.

Exon-trapping analysis

Exons are recognized by the splicing machinery through *cis*-acting regulatory sequences on the pre-mRNA and *trans*-acting factors associated with them, in addition to constitutive splicing factors defining each splice site [1].

To determine such *cis*-acting elements on the pre-mRNAs responsible for the splicing regulation of the picked-up examples in Fig. 1, exon-trapping vectors containing human genomic sequences around the alternatively spliced exons or their mutants were constructed, and the splicing of each transcript was examined in the tumor derived cell lines SW480 (Fig. 4). The spliced

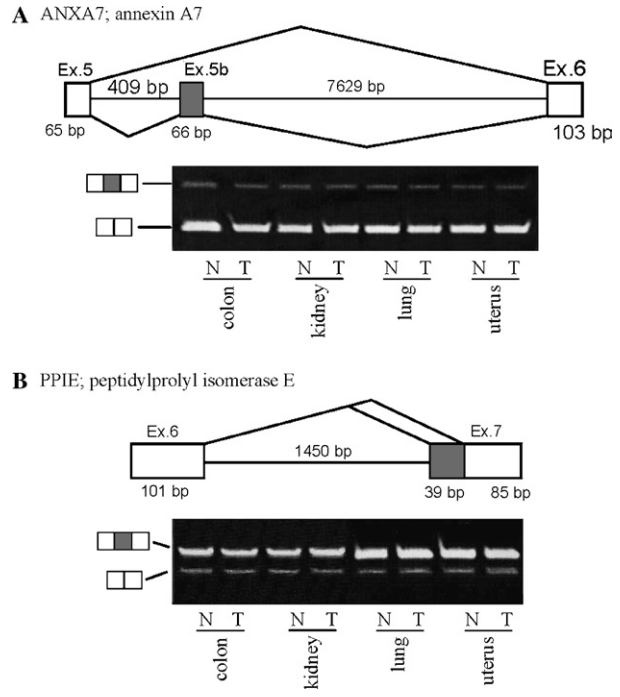

Fig. 3. Alternative splicing events not influenced in tumor tissues. For each indicated gene, the genomic structure around the alternatively spliced exons with the sizes of the exons and introns are shown above. The transcripts in the indicated tissues detected by RT-PCR are shown with its exon compositions below. The alternatively spliced exons are represented by black or gray boxes. N, normal tissue; T, tumor tissue.

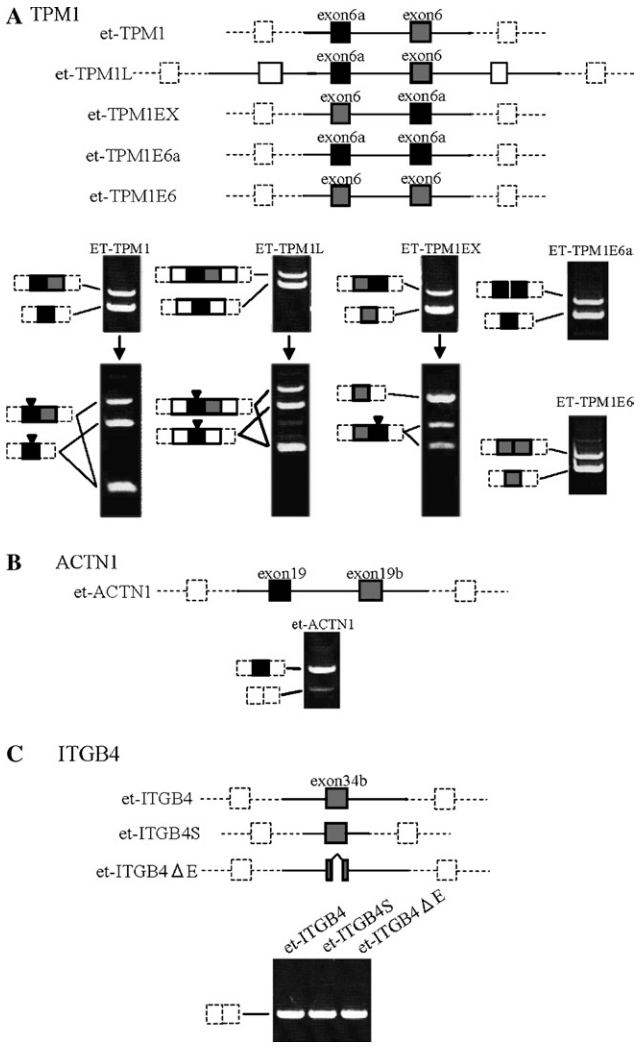

Fig. 4. Exon-trapping analyses. For each indicated gene, the constructs of the exon-trapping vectors and their derived transcripts in the tumor derived cell line SW480 detected by RT-PCR are shown. The lines and boxes represent the introns and exons of the genes, and their alternatively spliced exons are represented by black or gray boxes. The dotted lines and boxes represent the introns and exons derived from the vector. The exon compositions are indicated, and those of the et-TPM1 transcripts are determined by *Pvu*II digestion (arrows).

products detected by RT-PCR in the four cell lines (SW480, A549, Caki-1, and Hec-1) showed almost the same patterns (data not shown).

All the spliced products from the et-TPM1 vector contained exon 6a, consistent with the endogenous *TPM1* transcripts. However, a subset of the products also contained exon 6, and the pattern did not change with the et-TPM1L vector, which contained a larger amount of the genomic sequence. These results indicate there are *cis*-elements on the more distal area that suppress the inclusion of exon 6. Since the et-TPM1EX vector, in which exon 6a and 6 exchange their positions, lead to definite inclusion of exon 6 and part inclusion of exon 6a, this exon selection appeared to be regulated

by *cis*-elements on the intron in the et-TPM1EX vector, and to be independent of the exonic sequence. In addition, the et-TPM1E1 and et-TPM1E2 vectors, in which both exonic positions are occupied by the sequence for exon 6a or exon 6, respectively, showed similar distributions of the spliced products containing one or two exons. These observations support the hypothesis described above. A product with no exon, slightly detected in some cell lines (data not shown), indicated regulation by splicing enhancers on the distal area of the genome.

ACTN1 had exclusive alternatively spliced exons similar to *TPM1*, but the et-ACTN1 vector produced spliced products that either contained only exon 19 or had no exon. The lack of detection of exon 19b inclusion is consistent with the results for endogenous *ACTN1*, but not with those for the et-TPM1 vector, indicating different distributions of the splicing regulatory *cis*-elements on the genome for *ACTN1* and *TPM1*. A product with no exon, which was not detected in endogenous *ACTN1*, was detected more clearly than in et-TPM1 transcripts. These observations support the hypothesis of differential splicing regulation between *ACTN1* and *TPM1*.

With the et-ITGB4 vector, no exon inclusion was detected, similar to the endogenous *ITGB4* transcript. This result suggests that there are *cis*-elements for the endogenous *ITGB4* gene that suppress the inclusion of exon 34b in tumors or enhance its inclusion in normal tissue. The results for the et-ITGB4S and et-ITGB4ΔE vectors, for no exon inclusion were also detected, indicating that there is no suppressive *cis*-element at least in the deleted sequence in these constructs.

Discussion

Cancer-specific alternative splicing of many genes has previously been reported, and many researchers have suggested the importance of splicing regulation for cancer-specific protein expression [5,6]. However, the mechanism underlying cancer-specific splicing and the overall effect of alternative splicing on protein expression are poorly understood. The huge amount of available cDNA fragment data, which can be obtained from various recently established databases, makes it possible to predict unpublished alternative splicing events and calculate their expression specificities. In fact, some researchers have conducted such analyses and constructed an alternative splicing database. Furthermore, Lee et al. [18–22] also calculated the cancer specificity of each alternative splicing, although the obtained values are speculative and do not have an experimental basis.

We selected targets from the list of cancer-specific alternative splicing events calculated by Lee et al. [22], as well as alternative splicing reported to be cancer specific by other researchers [12–17] and some reported exclusive alternative splicing [15,23,24], and tried to confirm their expression specificities in tissue samples. Despite these targets being reported as cancer specific, our RT-PCR analysis revealed that only some of them were tumor tissue specific (21/31). This may be caused by the inaccurate information in the databases used by the other researchers, or different characters of the tumor tissue samples we used. The genes reported to show tumor-specific alternative splicing changed their expression patterns in many tumor samples, but not in all other tumor samples [12,16,17]. Among all the examined targets, exclusive alternative splicing events showed a high probability of tumor specificity (4/4), suggesting that the regulation of exclusive type alternative splicing is susceptible to tumorigenesis.

In the present study, endogenous *TPM1* exon 6, *ACTN1* exon 19b, and *ITGB4* exon 34b tended to be excluded in tumor tissues and cell lines, and the tendencies of all three examples were strongest in the uterus and weakest in the colon. There are two possibilities for the *cis*-elements regulating the tumor-specific suppression of exon inclusion, namely, suppression in tumor tissues and enhancement in normal tissues. The exon-trapping analyses for *TPM1* indicated there must be *cis*-elements in the distal region that suppress the inclusion of exon 6 in tumor tissues, which are not present in et-TPM1 and et-TPM1L, since exon 6 was partially included in transcripts from these vectors, but not from endogenous *TPM1*. However, since the inclusion of exon 6 was only partial, *cis*-elements suppressing the inclusion may also exist in these vectors. The results for et-ACTN1, et-ITGB4, et-ITGB4S, and et-ITGB4ΔE revealed no inclusion of exon 19b or exon 34b. These results suggest the existence of suppressive elements in these constructs that are active in tumor tissues, but do not eliminate the existence of enhancer elements in the distal region that are active in normal tissues. The presence of enhancer elements that are active in normal tissues requires further examination using primary cells or non-tumor cell lines, and the same is also true for the exclusiveness of *TPM1* and *ACTN1* splicing.

Despite their similar alterations of endogenous gene splicing, the different results obtained for et-TPM1 and et-ACTN1 indicate different distributions of splicing regulatory *cis*-elements on their pre-mRNAs, and the whole regulatory machinery that defines the different splicing patterns between normal and tumor tissues may also be unique for each gene. Hence, we can expect that there are multiple regulatory machineries that could be affected by tumorigenesis. The results for *NGLY1*, *p120ctn*, and *FGFR2* also support this hypothesis. For instance, in kidney tumors, *TPM1*, *ACTN1*, *ITGB4*, *p120ctn*, and *FGFR2* change their alternative splicing patterns, whereas *NGLY1* does not, despite its apparent alterations in lung and uterus tumors. These findings suggest that some factors are affected by the

tumorigenesis of one tissue, whereas other factors are affected by tumorigenesis in other tissues. The heterogeneous effects of tumorigenesis for the alternative splicing of many genes, as shown in 2 and Table 1, may indicate the complexity of tumor-specific splicing regulation.

In general, analysis of a number of tumor and normal samples and statistical data are needed to confirm tumor specificity [4]. The examples identified in the present study require such analyses for further confirmation, as well as identification of the active regulatory elements in normal tissues. However, the results of the current study provide key insights for elucidating the mechanisms underlying the cancer-specific protein expression.

Acknowledgment

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

[1] L.B. Douglas, Mechanism of alternative pre-messenger RNA splicing, Annu. Rev. Biochem. 72 (2003) 291–336.
[2] B. Modrek, C. Lee, A genomic view of alternative splicing, Nat. Genet. 30 (2002) 13–19.
[3] N.A. Faustino, T.A. Cooper, Pre-mRNA splicing and human disease, Genes Dev. 17 (2003) 419–437.
[4] J.P. Baak, F.R. Path, M.A. Hermsen, G. Meijer, J. Schmidt, E.A. Janssen, Genomics and proteomics in cancer, Eur. J. Cancer 39 (2003) 1199–1215.
[5] B.M. Brinkman, Splice variants as cancer biomarkers, Clin. Biochem. 37 (2004) 584–594.
[6] J.P. Venables, Aberrant and alternative splicing in cancer, Cancer Res. 64 (2004) 7647–7654.
[7] W. van der Houven van Oordt, M.T. Diaz-Meco, J. Lozano, A.R. Krainer, J. Moscat, J.F. Caceres, The MKK(3/6)-p38-signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation, J. Cell Biol. 149 (2000) 307–316.
[8] R. Daoud, G. Mies, A. Smialowska, L. Olah, K.A. Hossmann, S. Stamm, Ischemia induces a translocation of the splicing factor tra2-beta 1 and changes alternative splicing patterns in the brain, J. Neurosci. 22 (2002) 5889–5899.
[9] S. Weg-Remers, H. Ponta, P. Herrlich, H. Konig, Regulation of alternative pre-mRNA splicing by the ERK MAP-kinase pathway, EMBO J. 20 (2001) 4194–4203.
[10] E. Allemand, S. Guil, M. Myers, J. Moscat, J.F. Caceres, A.R. Krainer, Regulation of heterogenous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock, Proc. Natl. Acad. Sci. USA 102 (2005) 3605–3610.
[11] N. Shomron, M. Alberstein, M. Reznik, G. Ast, Stress alters the subcellular distribution of hSlu7 and thus modulates alternative splicing, J. Cell Sci. 118 (2005) 1151–1159.
[12] K.H. Heider, H. Kuthan, G. Stehle, G. Munzert, CD44v6: a target for antibody-based cancer therapy, Cancer Immunol. Immunother. 53 (2004) 567–579.
[13] S. Aho, L. Levansuo, O. Montonen, C. Kari, U. Rodeck, J. Uitto, Specific sequences in p120ctn determine subcellular distribution of its multiple isoforms involved in cellular adhesion of normal and malignant epithelial cells, J. Cell Sci. 115 (2002) 1391–1402.
[14] T. Tennenbaum, A.J. Belanger, A.B. Glick, R. Tamura, V. Quaranta, S.H. Yuspa, A splice variant of alpha 6 integrin is associated with malignant conversion in mouse skin tumorigenesis, Proc. Natl. Acad. Sci. USA 92 (1995) 7041–7045.
[15] K. Honda, T. Yamada, M. Seike, Y. Hayashida, M. Idogawa, T. Kondo, Y. Ino, S. Hirohashi, Alternative splice variant of actinin-4 in small cell lung cancer, Oncogene 23 (2004) 5257–5262.
[16] S.P. Gunningham, M.J. Currie, C. Han, B.A. Robinson, P.A. Scott, A.L. Harris, S.B. Fox, The short form of the alternatively spliced flt-4 but not its ligand vascular endothelial growth factor C is related to lymph node metastasis in human breast cancers, Clin. Cancer Res. 6 (2000) 4278–4286.
[17] Y. Tojo, S. Bandoh, J. Fujita, A. Kubo, T. Ishii, Y. Fukunaga, Y. Ueda, Y. Yang, F. Wu, C.L. Huang, H. Yokomise, T. Ishida, Aberrant messenger RNA splicing of the cytokeratin 8 in lung cancer, Lung Cancer 42 (2003) 153–161.
[18] L. Hui, X. Zhang, X. Wu, Z. Lin, Q. Wang, Y. Li, G. Hu, Identification of alternatively spliced mRNA variants related to cancers by genome-wide ESTs alignment, Oncogene 23 (2004) 3013–3023.
[19] H. Xie, W.Y. Zhu, A. Wasserman, V. Grebinskiy, A. Olson, L. Mintz, Computational analysis of alternative splicing using EST tissue information, Genomics 80 (2002) 326–330.
[20] Z. Wang, H.S. Lo, H. Yang, S. Gere, Y. Hu, K.H. Buetow, M.P. Lee, Computational analysis and experimental validation of tumor-associated alternative RNA splicing in human cancer, Cancer Res. 63 (2003) 655–657.
[21] Q. Xu, C. Lee, Discovery of novel splice forms and functional analysis of cancer-specific alternative splicing in human expressed sequences, Nucleic Acids Res. 31 (2003) 5635–5643.
[22] C. Lee, L. Atanelov, B. Modrek, Y. Xing, ASAP: the Alternative Splicing Annotation Project, Nucleic Acids Res. 31 (2003) 101–105.
[23] J. Kremerskothen, I. Teber, D. Wendholt, T. Liedtke, T.M. Bockers, A. Barnekow, Brain-specific splicing of alpha-actinin 1 (ACTN1) mRNA, Biochem. Biophys. Res. Commun. 295 (2002) 678–681.
[24] A. Orr-Urtreger, M.T. Bedford, T. Burakova, E. Arman, Y. Zimmer, A. Yayon, D. Givol, P. Lonai, Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2 (FGFR2), Dev. Biol. 158 (1993) 475–486.
[25] E. Perlino, M. Lovecchio, R.A. Vacca, M. Fornaro, L. Moro, P. Ditonno, M. Battaglia, F.P. Selvaggi, M.G. Mastropasqua, P. Bufo, L.R. Languino, Regulation of mRNA and protein levels of beta1 integrin variants in human prostate carcinoma, Am. J. Pathol. 157 (2000) 1727–1734.
[26] Y. Gokmen-Polar, N.R. Murray, M.A. Velasco, Z. Gatalica, A.P. Fields, Elevated protein kinase C betaII is an early promotive event in colon carcinogenesis, Cancer Res. 61 (2001) 1375–1381.
[27] L.C. Patten, N.S. Belaguli, M.J. Baek, S.P. Fagan, S.S. Awad, D.H. Berger, Serum response factor is alternatively spliced in human colon cancer, J. Surg. Res. 121 (2004) 92–100.