network among genes can be utilized for the estimation of a chemical's effect, from experimentally obtained expression profiles. The ability to identify expression profiles and the corresponding biological functions is expected to provide further possibilities for SEM in the inference of regulatory mechanisms by chemical toxicity.

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REFERENCES

[1] A. Baccarelli and V. Bollati, "Epigenetics and environmental chemicals," Curr. Opin. Pediatr. vol.21(2), pp. 243-251, Apr. 2009.

[2] L. Hou, X. Zhang, D. Wang, and A. Baccarelli, "Environmental chemical exposures and human epigenetics," Int. J. Epidemiol., vol. 41(1), pp. 79-105, Feb. 2012. doi:10.1093/ije/dyr154.

[3] Y. Yuan, "Methylmercury: a potential environmental risk factor contributing to epileptogenesis," Neurotoxicology, vol. 33(1), pp. 119-126, Jan. 2012.

[4] N. Tatsuta, et al., "Prenatal exposures to environmental chemicals and birth order as risk factors for child behavior problems," Environ. Res., vol.114, pp. 47-52, Apr. 2012.

[5] D. A. Rappolee, Y. Xie, J.A. Slater, S. Zhou, and E. E. Puscheck, "Toxic stress prioritizes and imbalances stem cell differentiation: implications for new biomarkers and in vitro toxicology tests," Syst. Biol. Reprod. Med., vol.58(1), pp. 33-40, Feb. 2012.

[6] X. He, et al., "Effects of methylmercury exposure on neuronal differentiation of mouse and human embryonic stem cells,". Toxicol. Lett., vol.212(1), pp. 1-10, Apr. 2012.

[7] J. A. Harrill, B. L. Robinette, and W. R. Mundy, "Use of high content image analysis to detect chemical-induced changes in synaptogenesis in vitro," Toxicol. In. Vitro., vol.25(1), pp. 368-387, Feb. 2011.

[8] U. Gündel, D. Benndorf, M. von Bergen, R. Altenburger, and E. Küster, "Vitellogenin cleavage products as indicators for toxic stress in zebra fish embryos: A proteomic approach," Proteomics, vol.7(24), pp. 4541-4554, Dec. 2007.

[9] J. Thompson and J.Bannigan, "Cadmium: toxic effects on the reproductive system and the embryo," Reprod. Toxicol., vol.25(3), pp. 304-315, Apr. 2008.

[10] T. Akutsu, S. Miyano, and S. Kuhara, "Algorithms for identifying Boolean networks and related biological networks based on matrix multiplication and fingerprint function," J. Comput. Biol., vol.7, pp. 331-343, 2000.

[11] N. Friedman, M. Linial, I. Nachman, and D. Pe'er, "Using Bayesian networks to analyze expression data," J. Comput. Biol., vol.7, pp. 601-620, 2000.

[12] S. Aburatani, S. Kuhara, H. Toh, and K. Horimoto, "Deduction of a gene regulatory relationship framework from gene expression data by the application of graphical Gaussian modeling," Signal Processing, vol.83, pp. 777-788, Apr. 2003.

[13] S. Aburatani and K. Horimoto, "Elucidation of the Relationships between LexA-Regulated Genes in the SOS response," Genome Informatics, vol.16(1), pp. 95-105, Aug. 2005.

[14] S. Aburatani, "Application of structure equation modeling for inferring a serial transcriptional regulation in yeast," Gene. Regul. Syst. Bio., vol.5, pp. 75-88, Nov. 2011.

[15] K. A. Bollen, Structural Equations with Latent Variables, New York: Wiley-Interscience, 1989.

[16] O. D. Duncan, Introduction to Structural Equation Models, New York: Academic Press, 1975.

[17] J. Pearl, Causality: Models, Reasoning, and Inference, 2nd ed., Cambridge: Cambridge University Press, 2001.

[18] W. Fujibuchi, et al., "Prediction of Chemical Toxicity by Network-based SVM on ES-cell Validation System," The Proc. of the 2011 Joint Conference of CBI-Society and JSBi, Nov. 2011.

[19] L. Li and G. E. Caldwell, "Coefficient of cross correlation and the time domain correspondence," J. of Electromyography and Kinesiology, vol.9, pp. 385–389, Dec. 1999.

[20] K. G. Joreskog and D. Sorbom, LISREL-VI: Analysis of Linear Structural Relationships By the Method of Maximum Likelihood, Redondo Beach: Doss-Haus Books, 1984.

[21] P. Spirtes, C. Glymour, and R. Scheines, Causation, Prediction, and Search, 2nd ed., Cambridge: The MIT Press, 2001.

[22] J. M. Rosenstein, N. Mani, A. Khaibullina, and J. M. Krum, "Neurotrophic effects of vascular endothelial growth factor on organotypic cortical explants and primary cortical neurons,". J. Neurosci., vol.23(35), pp. 11036-11044, Dec. 2003.

[23] N. Ito, et al., "Pathological markers for non-genotoxic agent-associated carcinogenesis,". Toxicol. Lett., Vol. 64-65, pp. 613-620, Dec. 1992.

[24] R. Puatanachokcha, et al., "Lack of promoting effects of phenobarbital at low dose on diethylnitrosamine-induced hepatocarcinogenesis in TGF-alpha transgenic mice," Asian Pac. J. Cancer Prev., vol.7(2), pp. 274-278, Apr-Jun 2006.

[25] M. J. Iatropoulos, C. X. Wang, K. E. von Keutz, and G. M. Williams, "Assessment of chronic toxicity and carcinogenicity in an accelerated cancer bioassay in rats of Nifurtimox, an antitrypanosomiasis drug," Exp. Toxicol. Pathol., vol.57(5-6), pp. 397-404, Jul. 2006.

[26] H. Hamada, C. Meno, D. Watanabe, and Y. Saijoh, "Establishment of vertebrate left-right asymmetry," Nat. Rev. Genet., vol.3(2), pp. 103-113, Feb. 2002.

[27] C. Grandel and N. H. Patel, "Nodal signaling is involved in left-right asymmetry in snails," Nature, vol.457(7232), pp. 1007–1011, Feb. 2009.

[28] K. Mitsui, "The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells," Cell, vol.113(5), pp. 631-642, May 2003.

[29] I. Chambers, et al., "Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells," Cell, vol.113(5), pp. 643-655, May 2003.

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REVIEW

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 VEGFAxxx and VEGFAxxxb, which have proangiogenic and antiangiogenic properties, respectively; *UGT1A* is alternatively spliced into UGT1A1 and other isoforms, which are regulated by pregnane 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 (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. Another recent study 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 subclassified 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 multicomponent 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

Abbreviations: 5-FU, 5-fluorouracil; 2′-OMe, 2′-O-methyl; 2′-MOE, 2′-O-methoxyethyl; 3′SS, 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; CRNDE, 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, intronic splice enhancer; ISS, intronic splice silencer; pre-mRNA, precursor messenger RNA; PXR, pregnane X receptor; siRNA, small interfering RNA; SR protein, serine/arginine-rich protein; UGT1A1, UDP glucuronosyltransferase 1A1; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

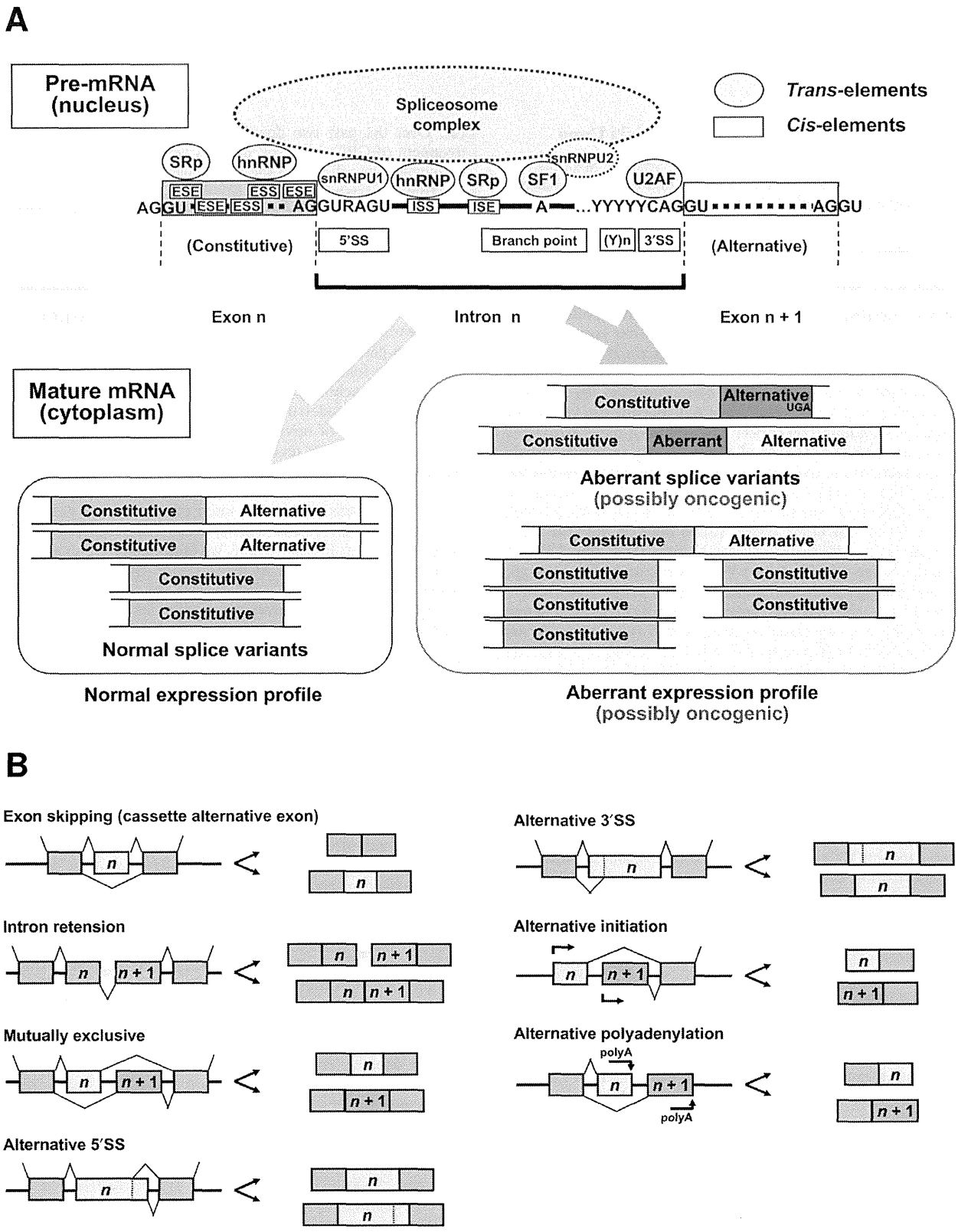

Fig. 1. (**A**) The regulation of alternative pre-mRNA splicing and its alteration in malignancies (adapted from ref. 3). *Cis*-elements and *trans*-elements are indicated with rectangles and ellipses, respectively. In the nucleotide sequences, Y denotes a pyrimidine (U or C) and R denotes a purine (G 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 VEGFA binding to VEGFR-1 and VEGFR-2 (13). VEGFA 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 8 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 VEGFAxxx subfamily have been explored and pegaptanib was developed as a short modified RNA aptamer that specifically binds to VEGFAxxx but not VEGFAxxxb (20). Another possible target for CRC is a trans-element, SRp55, which is known to increase VEGFAxxxb expression, leading to antiangiogenic effects (21). In addition, IGF1, 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 prodrug 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 RBM8A, 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 G_1-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 (CC**G** and CC**A**) and is located adjacent to the 5′SS of intron 4 (**GU**RAGU 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

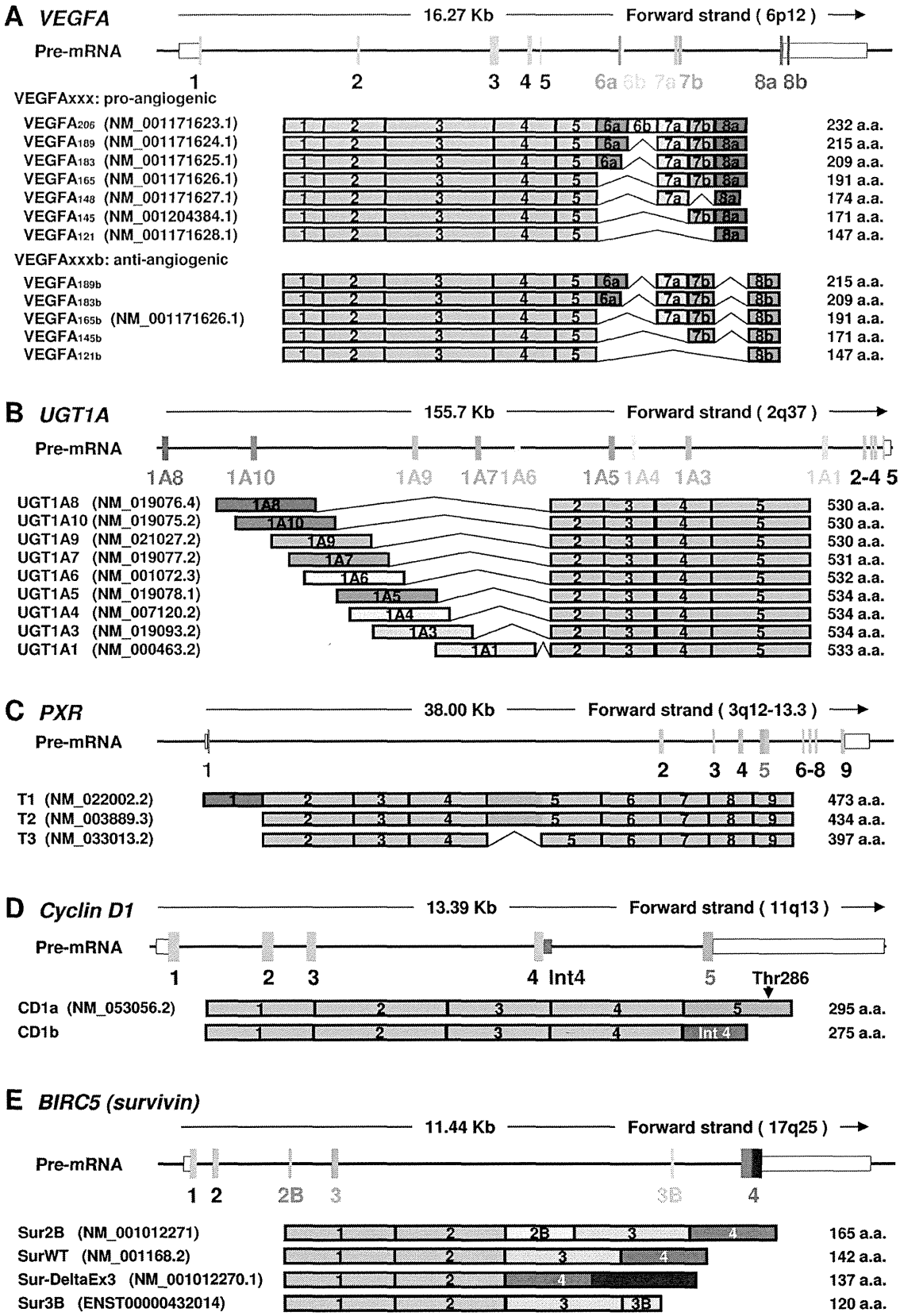

Fig. 2. Splice isoforms of *VEGFA* (**A**), *UGT1A* (**B**), *PXR* (**C**), *cyclin D1* (**D**) and *survivin* (**E**). For each of the genes, the pre-mRNA is indicated at the top and mature mRNAs are indicated below. White boxes indicate 5′-UTR and 3′-UTR. The NM numbers and the numbers of amino acids were provided based on the information contained in the NCBI database (as of 30 September 2012).

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 catabolized by dihydropyrimidine dehydrogenase (DPD), mainly in the liver, whereas the remaining 10% of 5-FU is anabolized to exert cytotoxic activity (61), making DPD the most important determinant of 5-FU metabolism (62). Screening for genetic alterations with genomic DNA and mRNA sequencing, van Kuilenburg *et al.* (63) identified genetic alterations in deep intronic regions such as c.1129-5923C>G, which caused aberrant splice isoforms of the *DPD* gene. Their study indicates that caution should be exercised when screening for introns, as well as for exons, when identifying DPD-deficient patients and determining the likely efficacy of 5-FU.

Other splice isoforms implicated in CRC

Some of the other genes encoding splice isoforms that may be possible therapeutic targets for CRC are the *K-RAS* (64), *macroH2A1* (65,66), *SOX9* (67), *SLC39A14* (68,69), colorectal neoplasia differentially expressed (*CRNDE*) (70), *BARD1* (71), *CDH17* (72), *CYP24A1* (73) and *PPARG* genes (74). It is well known that somatic mutation of the *K-RAS* gene is an early event in colorectal carcinogenesis. However, since the middle of the 2000s, the splice isoforms K-RAS4A and K-RAS4B have been reported to have differential functions in apoptosis (75) and differentiation (76) in the intestinal epithelia. In 2009, Abubaker *et al.* (64) analyzed CRC tissues for somatic mutations in the *K-RAS* gene, as well as performing an immunohistochemical analysis of the splice isoforms. Their study demonstrated that the expression of K-RAS4A and K-RAS4B was associated with several clinicopathological features of CRC, and both the *K-RAS* mutation and K-RAS4A expression were independent prognostic markers in a multivariate analysis.

MacroH2A1 is the founding member of the macroH2As family, which has the ability to replace the functions of canonical histones, and has two splice isoforms: macroH2A1.1 and macroH2A1.2. In 2011, Novikov *et al.* (65) demonstrated that the expression of macroH2A1.1 is suppressed in CRC and other malignancies compared with normal tissues. An immunohistochemical study of the two isoforms by Sporn *et al.* (66) in 2012 revealed that the loss of macroH2A1.1 was associated with a worse prognosis of CRC.

The SOX9 transcription factor, which has antioncogenic potential in CRC, generates two isoforms: canonical SOX9 and MiniSOX9, which is a truncated isoform of SOX9 expressed at high levels in CRC (67). An immunohistochemical analysis of CRC and corresponding normal tissues using isoform-specific antibodies revealed that MiniSOX9 behaves as a SOX9 inhibitor and increases the oncogenic potential of CRC cells (67). This indicates that MiniSOX9 may be a therapeutic target for CRC.

SLC39A14 is a divalent cation transporter, which consists of nine exons and has two splice isoforms with a mutually exclusive exon 4, which generates two isoforms: SLC39A14-4A and SLC39A14-4B. In 2011, Thorsen *et al.* (69) demonstrated that SLC39A14-4B mRNA is highly expressed in colonic adenoma and CRC tissues compared with the SLC39A14-4A mRNA. In 2011, Sveen *et al.* (68) reported that SLC39A14-4B can be used as a marker to distinguish CRC from other pathological conditions of the colon. In addition, Graham *et al.* (70) indicated that splice isoforms of the *CRNDE* gene seem to be differentially expressed in different stages of CRC.

In 2011, Yi and Tang reported a review article on the potential use of splice variants as diagnostic, predictive and prognostic markers for CRC (77), which included information about *APC*, *TIMP-1*, *VEGFA*, *DYX1C1* and *c-FLIP*, among other genes. Their article provided information about the splice isoforms with regard to the use of chemotherapy for CRC. A genome-wide exon array analysis in 2011 detected several CRC-specific splice isoforms (*TCF12*, *OSBPL1A*, *TRAK1*, *ANK3*, *CHEK1*, *UGP2*, *LMO7*, *ACSL5* and *SCIN*) (78). In addition, trans-elements, such as SR protein kinase 1 and SR protein kinase 2, have also been discussed as therapeutic targets for CRC and other malignancies (79), although trans-elements are not described in detail in this review. Most of the studies presented here were reported after 2011, and the information on splice isoforms is still being accumulated.

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

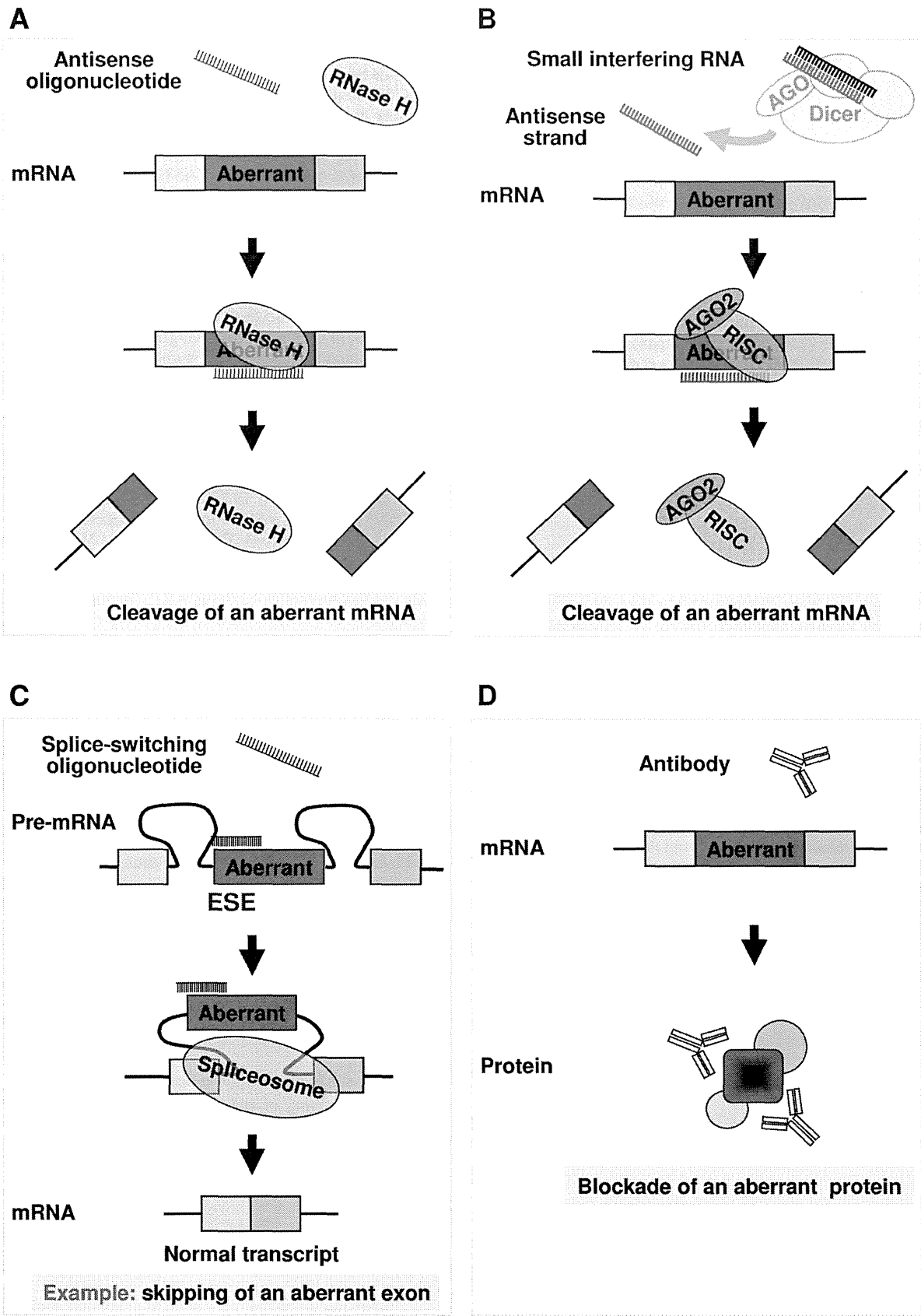

Fig. 3. Macromolecules targeting splice isoforms associated with malignancy. (**A**) A synthetically modified antisense oligonucleotide, (**B**) siRNA, (**C**) a splice-switching oligonucleotide and (**D**) an antibody. In (**C**), an example of splice switching is presented, in which an ESE located 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.

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 argonaute 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 coordinated 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 (MMBGX) 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.

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References

1. Jemal,A. *et al.* (2011) Global cancer statistics. *CA. Cancer J. Clin.*, **61**, 69–90.
2. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site
3. Miura,K. *et al.* (2011) Alternative pre-mRNA splicing in digestive tract malignancy. *Cancer Sci.*, **102**, 309–316.
4. Wang,E.T. *et al.* (2008) Alternative isoform regulation in human tissue transcriptomes. *Nature*, **456**, 470–476.
5. Yoshida,K. *et al.* (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*, **478**, 64–69.
6. Moore,M.J. *et al.* (2010) An alternative splicing network links cell-cycle control to apoptosis. *Cell*, **142**, 625–636.
7. Chow,L.T. *et al.* (1977) An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA. *Cell*, **12**, 1–8.
8. Klessig,D.F. (1977) Two adenovirus mRNAs have a common 5' terminal leader sequence encoded at least 10kb upstream from their main coding regions. *Cell*, **12**, 9–21.
9. Chen,M. *et al.* (2009) Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches. *Nat. Rev. Mol. Cell Biol.*, **10**, 741–754.
10. Warzecha,C.C. *et al.* (2009) ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing. *Mol. Cell*, **33**, 591–601.
11. Luco,R.F. *et al.* (2011) Epigenetics in alternative pre-mRNA splicing. *Cell*, **144**, 16–26.
12. Amankwah,E.K. *et al.* (2012) Gene variants in the angiogenesis pathway and prostate cancer. *Carcinogenesis*, **33**, 1259–1269.
13. Rennel,E.S. *et al.* (2009) Therapeutic potential of manipulating VEGF splice isoforms in oncology. *Future Oncol.*, **5**, 703–712.
14. Bates,D.O. *et al.* (2002) VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. *Cancer Res.*, **62**, 4123–4131.
15. Harper,S.J. *et al.* (2008) VEGF-A splicing: the key to anti-angiogenic therapeutics? *Nat. Rev. Cancer*, **8**, 880–887.
16. Rennel,E.S. *et al.* (2009) VEGF(121)b, a new member of the VEGF(xxx)b family of VEGF-A splice isoforms, inhibits neovascularisation and tumour growth *in vivo*. *Br. J. Cancer*, **101**, 1183–1193.
17. Presta,L.G. *et al.* (1997) Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res.*, **57**, 4593–4599.
18. Muller,Y.A. *et al.* (1997) Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 7192–7197.
19. Troiani,T. *et al.* (2012) Beyond bevacizumab: new anti-VEGF strategies in colorectal cancer. *Expert Opin. Investig. Drugs*, **21**, 949–959.
20. Gragoudas,E.S. *et al.*; VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. (2004) Pegaptanib for neovascular age-related macular degeneration. *N. Engl. J. Med.*, **351**, 2805–2816.
21. Nowak,D.G. *et al.* (2008) Expression of pro- and anti-angiogenic isoforms of VEGF is differentially regulated by splicing and growth factors. *J. Cell. Sci.*, **121**(Pt 20), 3487–3495.
22. Haaz,M.C. *et al.* (1998) Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. *Cancer Res.*, **58**, 468–472.
23. Rivory,L.P. *et al.* (1997) Pharmacokinetic interrelationships of irinotecan (CPT-11) and its three major plasma metabolites in patients enrolled in phase I/II trials. *Clin. Cancer Res.*, **3**, 1261–1266.

24. Ando,Y. *et al.* (2000) Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res.*, **60**, 6921–6926.
25. Sai,K. *et al.* (2004) UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. *Clin. Pharmacol. Ther.*, **75**, 501–515.
26. Innocenti,F. *et al.* (2004) Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J. Clin. Oncol.*, **22**, 1382–1388.
27. Gong,Q.H. *et al.* (2001) Thirteen UDPglucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. *Pharmacogenetics*, **11**, 357–368.
28. Ritter,J.K. *et al.* (1992) A novel complex locus UGT1 encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini. *J. Biol. Chem.*, **267**, 3257–3261.
29. Girard,H. *et al.* (2007) Genetic diversity at the UGT1 locus is amplified by a novel 3' alternative splicing mechanism leading to nine additional UGT1A proteins that act as regulators of glucuronidation activity. *Pharmacogenet. Genomics*, **17**, 1077–1089.
30. Bellemare,J. *et al.* (2011) Immunohistochemical expression of conjugating UGT1A-derived isoforms in normal and tumoral drug-metabolizing tissues in humans. *J. Pathol.*, **223**, 425–435.
31. Bellemare,J. *et al.* (2010) Alternatively spliced products of the UGT1A gene interact with the enzymatically active proteins to inhibit glucuronosyltransferase activity *in vitro*. *Drug Metab. Dispos.*, **38**, 1785–1789.
32. Raynal,C. *et al.* (2010) Pregnane X Receptor (PXR) expression in colorectal cancer cells restricts irinotecan chemosensitivity through enhanced SN-38 glucuronidation. *Mol. Cancer*, **9**, 46.
33. Gardner-Stephen,D. *et al.* (2004) Human PXR variants and their differential effects on the regulation of human UDP-glucuronosyltransferase gene expression. *Drug Metab. Dispos.*, **32**, 340–347.
34. Solier,S. *et al.* (2010) Genome-wide analysis of novel splice variants induced by topoisomerase I poisoning shows preferential occurrence in genes encoding splicing factors. *Cancer Res.*, **70**, 8055–8065.
35. Lew,D.J. *et al.* (1991) Isolation of three novel human cyclins by rescue of G1 cyclin (Cln) function in yeast. *Cell*, **66**, 1197–1206.
36. Arber,N. *et al.* (1996) Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. *Gastroenterology*, **110**, 669–674.
37. Wang,Q.S. *et al.* (1998) Altered expression of cyclin D1 and cyclin-dependent kinase 4 in azoxymethane-induced mouse colon tumorigenesis. *Carcinogenesis*, **19**, 2001–2006.
38. Jiang,W. *et al.* (1993) Altered expression of the cyclin D1 and retinoblastoma genes in human esophageal cancer. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 9026–9030.
39. Zwijsen,R.M. *et al.* (1997) CDK-independent activation of estrogen receptor by cyclin D1. *Cell*, **88**, 405–415.
40. Li,Z. *et al.* (2010) Alternative cyclin D1 splice forms differentially regulate the DNA damage response. *Cancer Res.*, **70**, 8802–8811.
41. Witzel,I.I. *et al.* (2010) Regulation of cyclin D1 gene expression. *Biochem. Soc. Trans.*, **38**, 217–222.
42. Knudsen,K.E. (2006) The cyclin D1b splice variant: an old oncogene learns new tricks. *Cell Div.*, **1**, 15.
43. Betticher,D.C. *et al.* (1995) Alternate splicing produces a novel cyclin D1 transcript. *Oncogene*, **11**, 1005–1011.
44. Knudsen,K.E. *et al.* (2006) Cyclin D1: polymorphism, aberrant splicing and cancer risk. *Oncogene*, **25**, 1620–1628.
45. Olshavsky,N.A. *et al.* (2010) Identification of ASF/SF2 as a critical, allele-specific effector of the cyclin D1b oncogene. *Cancer Res.*, **70**, 3975–3984.
46. Paronetto,M.P. *et al.* (2010) Alternative splicing of the cyclin D1 proto-oncogene is regulated by the RNA-binding protein Sam68. *Cancer Res.*, **70**, 229–239.
47. Solomon,D.A. *et al.* (2003) Cyclin D1 splice variants. Differential effects on localization, RB phosphorylation, and cellular transformation. *J. Biol. Chem.*, **278**, 30339–30347.
48. Hinds,M.G. *et al.* (1999) Solution structure of a baculoviral inhibitor of apoptosis (IAP) repeat. *Nat. Struct. Biol.*, **6**, 648–651.
49. Sun,C. *et al.* (1999) NMR structure and mutagenesis of the inhibitor-of-apoptosis protein XIAP. *Nature*, **401**, 818–822.
50. Miura,K. *et al.* (2009) cIAP2 as a therapeutic target in colorectal cancer and other malignancies. *Expert Opin. Ther. Targets*, **13**, 1333–1345.
51. Nakamura,N. *et al.* (2012) ILF3/NF110 is a target of YM155, a suppressant of survivin. *Mol. Cell. Proteomics*, **11**, M111.013243.
52. Carrasco,R.A. *et al.* (2011) Antisense inhibition of survivin expression as a cancer therapeutic. *Mol. Cancer Ther.*, **10**, 221–232.
53. Chang,W.H. *et al.* (2011) Amiloride modulates alternative splicing in leukemic cells and resensitizes Bcr-AblT315I mutant cells to imatinib. *Cancer Res.*, **71**, 383–392.
54. Sampath,J. *et al.* (2007) Alternative splice variants of survivin as potential targets in cancer. *Curr. Drug Discov. Technol.*, **4**, 174–191.
55. Sawai,K. *et al.* (2010) Survivin-3B gene decreases the invasion-inhibitory effect of colon cancer cells with 5-fluorouracil. *Oncol. Res.*, **18**, 541–547.
56. Huang,Y. *et al.* (2011) Expression and prognostic significance of survivin splice variants in diffusely infiltrating astrocytoma. *J. Clin. Pathol.*, **64**, 953–959.
57. Vivas-Mejia,P.E. *et al.* (2011) Silencing survivin splice variant 2B leads to antitumor activity in taxane–resistant ovarian cancer. *Clin. Cancer Res.*, **17**, 3716–3726.
58. Pavlidou,A. *et al.* (2011) Survivin isoforms and clinicopathological characteristics in colorectal adenocarcinomas using real-time qPCR. *World J. Gastroenterol.*, **17**, 1614–1621.
59. Antonacopoulou,A.G. *et al.* (2011) The survivin -31 snp in human colorectal cancer correlates with survivin splice variant expression and improved overall survival. *Cell. Oncol. (Dordr).*, **34**, 381–391.
60. Heidelberger,C. *et al.* (1957) Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature*, **179**, 663–666.
61. Heggie,G.D. *et al.* (1987) Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res.*, **47**, 2203–2206.
62. Miura,K. *et al.* (2012) S-1 as a core anticancer fluoropyrimidine agent. *Expert Opin. Drug Deliv.*, **9**, 273–286.
63. van Kuilenburg,A.B. *et al.* (2010) Intragenic deletions and a deep intronic mutation affecting pre-mRNA splicing in the dihydropyrimidine dehydrogenase gene as novel mechanisms causing 5-fluorouracil toxicity. *Hum. Genet.*, **128**, 529–538.
64. Abubaker,J. *et al.* (2009) Prognostic significance of alterations in KRAS isoforms KRAS-4A/4B and KRAS mutations in colorectal carcinoma. *J. Pathol.*, **219**, 435–445.
65. Novikov,L. *et al.* (2011) QKI-mediated alternative splicing of the histone variant MacroH2A1 regulates cancer cell proliferation. *Mol. Cell Biol.*, **31**, 4244–4255.
66. Sporn,J.C. *et al.* (2012) Differential regulation and predictive potential of MacroH2A1 isoforms in colon cancer. *Am. J. Pathol.*, **180**, 2516–2526.
67. Abdel-Samad,R. *et al.* (2011) MiniSOX9, a dominant-negative variant in colon cancer cells. *Oncogene*, **30**, 2493–2503.
68. Sveen,A. *et al.* (2012) The exon-level biomarker SLC39A14 has organ-confined cancer-specificity in colorectal cancer. *Int. J. Cancer*, **131**, 1479–1485.
69. Thorsen,K. *et al.* (2011) Alternative splicing of SLC39A14 in colorectal cancer is regulated by the Wnt pathway. *Mol. Cell Proteomics*, **10**, M110.002998.
70. Graham,L.D. *et al.* (2011) Colorectal neoplasia differentially expressed (CRNDE), a novel gene with elevated expression in colorectal adenomas and adenocarcinomas. *Genes Cancer*, **2**, 829–840.
71. Sporn,J.C. *et al.* (2011) BARD1 expression predicts outcome in colon cancer. *Clin. Cancer Res.*, **17**, 5451–5462.
72. Lee,N.P. *et al.* (2010) Role of cadherin-17 in oncogenesis and potential therapeutic implications in hepatocellular carcinoma. *Biochim. Biophys. Acta*, **1806**, 138–145.
73. Horváth,H.C. *et al.* (2010) CYP24A1 splice variants–implications for the antitumorigenic actions of 1,25-(OH)2D3 in colorectal cancer. *J. Steroid Biochem. Mol. Biol.*, **121**, 76–79.
74. Koh,W.P. *et al.* (2006) Peroxisome proliferator-activated receptor (PPAR) gamma gene polymorphisms and colorectal cancer risk among Chinese in Singapore. *Carcinogenesis*, **27**, 1797–1802.
75. Plowman,S.J. *et al.* (2006) The K-Ras 4A isoform promotes apoptosis but does not affect either lifespan or spontaneous tumor incidence in aging mice. *Exp. Cell Res.*, **312**, 16–26.
76. Patek,C.E. *et al.* (2008) The pro-apoptotic K-Ras 4A proto-oncoprotein does not affect tumorigenesis in the ApcMin/+ mouse small intestine. *BMC Gastroenterol.*, **8**, 24.
77. Yi,Q. *et al.* (2011) Alternative spliced variants as biomarkers of colorectal cancer. *Curr. Drug Metab.*, **12**, 966–974.
78. Thorsen,K. *et al.* (2011) Tumor-specific usage of alternative transcription start sites in colorectal cancer identified by genome-wide exon array analysis. *BMC Genomics*, **12**, 505.
79. Hayes,G.M. *et al.* (2007) Serine-arginine protein kinase 1 overexpression is associated with tumorigenic imbalance in mitogen-activated protein kinase pathways in breast, colonic, and pancreatic carcinomas. *Cancer Res.*, **67**, 2072–2080.
80. Kole,R. *et al.* (2012) RNA therapeutics: beyond RNA interference and antisense oligonucleotides. *Nat. Rev. Drug Discov.*, **11**, 125–140.
81. Deleavey,G.F. *et al.* (2012) Designing chemically modified oligonucleotides for targeted gene silencing. *Chem. Biol.*, **19**, 937–954.
82. Pillay,V. *et al.* (2011) Antibodies in oncology. *N. Biotechnol.*, **28**, 518–529.

83. Tabrizi,M.A. *et al.* (2009) Translational strategies for development of monoclonal antibodies from discovery to the clinic. *Drug Discov. Today*, **14**, 298–305.
84. Barash,Y. *et al.* (2010) Deciphering the splicing code. *Nature*, **465**, 53–59.
85. Irimia,M. *et al.* (2012) Alternative splicing: decoding an expansive regulatory layer. *Curr. Opin. Cell Biol.*, **24**, 323–332.
86. Montes,M. *et al.* (2012) Functional coupling of transcription and splicing. *Gene*, **501**, 104–117.
87. Luco,R.F. *et al.* (2011) More than a splicing code: integrating the role of RNA, chromatin and non-coding RNA in alternative splicing regulation. *Curr. Opin. Genet. Dev.*, **21**, 366–372.
88. de Almeida,S.F. *et al.* (2012) Design principles of interconnections between chromatin and pre-mRNA splicing. *Trends Biochem. Sci.*, **37**, 248–253.
89. Luco,R.F. *et al.* (2010) Regulation of alternative splicing by histone modifications. *Science*, **327**, 996–1000.
90. McManus,C.J. *et al.* (2011) RNA structure and the mechanisms of alternative splicing. *Curr. Opin. Genet. Dev.*, **21**, 373–379.
91. Will,C.L. *et al.* (2011) Spliceosome structure and function. *Cold Spring Harb. Perspect. Biol.*, **3**, pii: a003707.
92. Gromak,N. (2012) Intronic microRNAs: a crossroad in gene regulation. *Biochem. Soc. Trans.*, **40**, 759–761.
93. Esteller,M. (2011) Non-coding RNAs in human disease. *Nat. Rev. Genet.*, **12**, 861–874.
94. Turro,E. *et al.* (2010) MMBGX: a method for estimating expression at the isoform level and detecting differential splicing using whole-transcript Affymetrix arrays. *Nucleic Acids Res.*, **38**, e4.

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REVIEW ARTICLE

Differentiating rectal carcinoma by an immunohistological analysis of carcinomas of pelvic organs based on the NCBI Literature Survey and the Human Protein Atlas database

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Abstract The treatments and prognoses of pelvic organ carcinomas differ, depending on whether the primary tumor originated in the rectum, urinary bladder, prostate, ovary, or uterus; therefore, it is essential to diagnose pathologically the primary origin and stages of these tumors. To establish the panels of immunohistochemical markers for differential diagnosis, we reviewed 91 of the NCBI articles on these topics and found that the results correlated closely with those of the public protein database, the Human Protein Atlas. The results revealed the panels of immunohistochemical markers for the differential diagnosis of rectal adenocarcinoma, in which [+] designates positivity in rectal adenocarcinoma and [−] designates negativity in rectal adenocarcinoma: from bladder adenocarcinoma, CDX2[+], VIL1[+], KRT7[−], THBD[−] and UPK3A[−]; from prostate adenocarcinoma, CDX2[+], VIL1[+], CEACAM5[+], KLK3(PSA)[−], ACPP(PAP)[−] and SLC45A3(prostein)[−]; and from ovarian mucinous adenocarcinoma, CEACAM5[+], VIL1[+], CDX2[+], KRT7[−] and MUC5AC[−]. The panels of markers distinguishing ovarian serous adenocarcinoma, cervical carcinoma, and endometrial adenocarcinoma were also represented. Such a comprehensive review on the differential diagnosis of carcinomas of pelvic organs has not been reported before. Thus, much information has been accumulated in public databases to provide an invaluable resource for clinicians and researchers.

Keywords Rectal adenocarcinoma · Carcinoma of pelvic organs · Differential diagnosis · Immunohistochemistry · Public database

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Abbreviations

IHC	Immunohistochemistry
H–E	Hematoxylin and eosin
LNs	Lymph nodes
HPA	Human Protein Atlas
NCBI	National Center for Biotechnology Information
HPR	Human Proteome Resource
KRT	Keratin
IF	Intermediate filament
CEA	Carcinoembryonic antigen
CEACAM5	Carcinoembryonic antigen-related cell adhesion molecule 5
CDX2	Caudal type homeobox 2
VIL1	Villin 1
THBD	Thrombomodulin

UPK3A	Uroplakin 3A
KLK3	Kallikrein-related peptidase 3
PSA	Prostate-specific antigen
ACPP	Acid phosphatase, prostate
PAP	Prostatic acid phosphatase
SLC45A3	Solute carrier family 45, member 3
MUC5AC	Mucin 5AC, oligomeric mucus/gel-forming
WT	Wilms' tumor
CDKN2A	Cyclin-dependent kinase inhibitor 2A
HPV	Human papillomavirus
VIM	Vimentin
ESR1	Estrogen receptor 1
PR	Progesterone receptor

Introduction

Establishing the origin of primary tumors or metastatic cancer cells from carcinomas of pelvic organs is often difficult preoperatively and intraoperatively. It is essential to diagnose the primary origin and tumor stage pathologically because treatment selection and prognosis differ according to the organ of origin, namely, the rectum, urinary bladder, prostate, ovary, or uterus. It is important to use immunohistochemistry (IHC) to support the pathological diagnosis with hematoxylin and eosin (H–E) staining when determining the primary origin and stage. IHC helps to reduce the rate of false-negative and false-positive diagnoses, and the knowledge of IHC is constantly expanding. Therefore, it would be beneficial to integrate all of the current knowledge on IHC markers for the differential diagnosis of carcinomas of the pelvic organs.

None of the IHC markers is absolutely sensitive or specific for a particular tumor type because tumors often show aberrant expression of proteins; hence, a panel of IHC markers is essential. Figure 1 illustrates a recent case in which the diagnosis of the primary origin of metastatic cancer cells was important. A concurrent diagnosis of prostate carcinoma was made in a patient with rectal adenocarcinoma and postoperative pathological examination revealed that some of the lymph nodes (LNs) were metastatic from rectal adenocarcinoma and others were from prostate adenocarcinoma (Fig. 1c–g). Based on these findings, androgen deprivation therapy (leuprorelin acetate) and systemic chemotherapy, including oxaliplatin, were introduced postoperatively. This case highlights that standardized panels of IHC markers need to be established for an accurate diagnosis in such cases. Another case report described a giant T4 rectal adenocarcinoma mimicking urinary bladder adenocarcinoma in a patient who underwent surgery after the primary origin was identified by IHC [1]. In this case, an incorrect diagnosis by IHC may have led to improper treatment. In yet another report, IHC was found to be important for the diagnosis of endoluminal metastasis of colon cancer via the ureter [2].

Public databases have recently accumulated much information for clinicians and basic researchers. The public protein database, Human Protein Atlas (HPA), provides a powerful strategy for diagnoses with pathological images and clinicopathological information from a wide variety of normal tissues, cell lines, and cancer tissues [3]. We surveyed literature from the National Center for Biotechnology Information (NCBI) PubMed database and reviewed approximately 600 related NCBI articles. From these, we selected 91 key studies and integrated their data into one summary, which was validated utilizing the HPA database. This review identifies panels of IHC markers for the differential diagnosis of rectal adenocarcinoma from carcinomas of other pelvic organs, including the urinary bladder, prostate, ovary, and uterus. These panels will be helpful not only for general surgeons, but also for urologists and gynecologists. To our knowledge, such a comprehensive review on the differential diagnosis of carcinomas of pelvic organs has never been reported before, from any country.

Review of the NCBI database

In December 2010, we selected approximately 200 articles in the first round of the NCBI PubMed literature survey on the IHC of carcinomas of the rectum and other pelvic organs, using the following keywords: "differential diagnosis" and "rectal adenocarcinoma" or "pelvic carcinoma", and "immunohistochemistry". This process identified 24 IHC markers (Table 1). We then conducted a second literature survey using the following key words: "official symbol" or "other aliases" and "organ name" and "carcinoma" and "immunohistochemistry"; in which, the "official symbol" and "other aliases" are listed in Table 1, and "organ name" was either "rectal", "bladder", "urothelial", "prostate", "ovary", "cervical" or "endometrial". This revealed approximately 600 references related to IHC of carcinomas of pelvic organs, but they did not necessarily include information for the current study. These references were carefully checked, and the 91 key articles were selected. We evaluated these 91 studies and integrated their data into Fig. 2 [4–94].

Guidelines for immunoreactivity

In Fig. 2, each of the 91 references is indicated by a leading number for all cells. We used the guidelines proposed by Hammerich et al. [32] to standardize the immunoreactivity

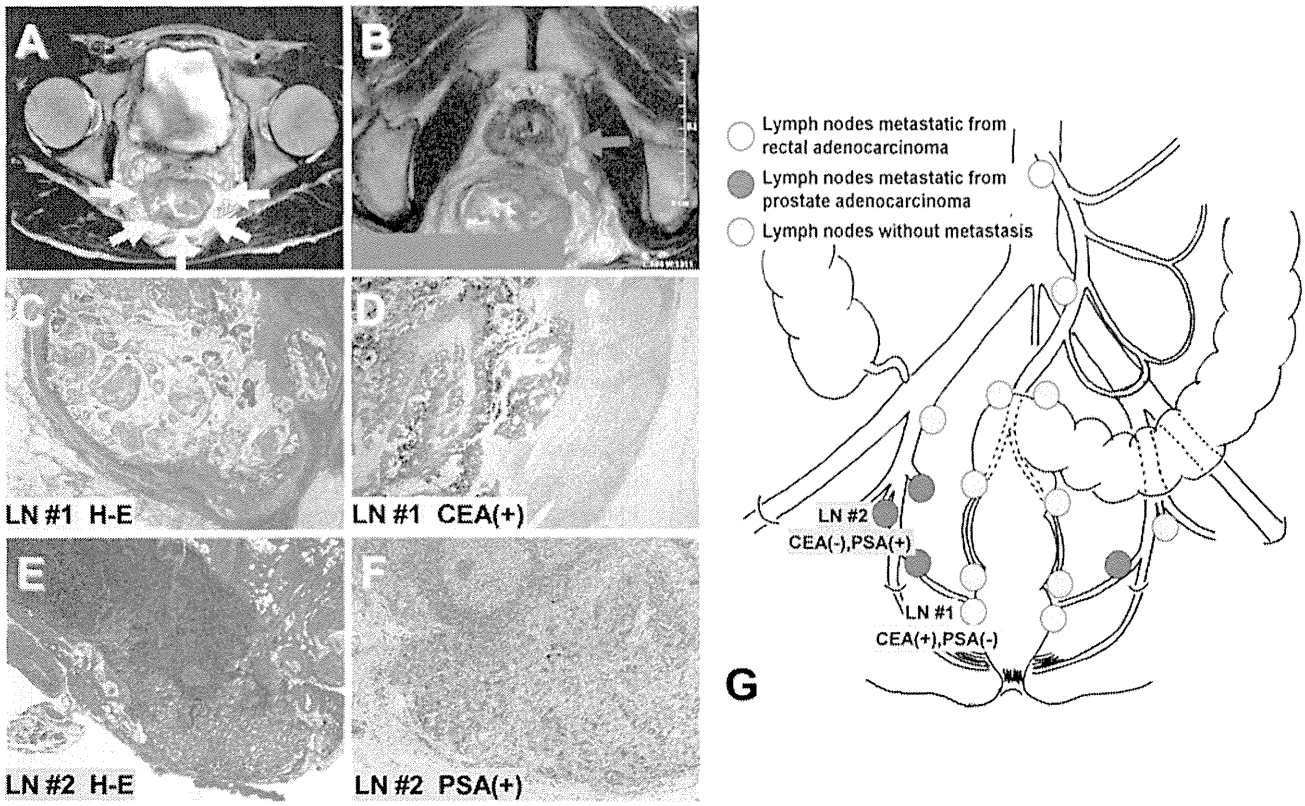

Fig. 1 Synchronous double carcinomas of the prostate and colorectum with lymph node metastasis. **a** Magnetic resonance imaging (MRI) axial image showing the rectal adenocarcinoma (*yellow arrows*). **b** MRI axial image showing the prostate adenocarcinoma (*purple arrows*). A metastatic LN in the pararectal region with H–E staining (**c**) and immunohistochemistry (IHC) for carcinoembryonic antigen (*CEA*) (**d**), showing possible rectal adenocarcinoma origin. A metastatic lymph node (*LN*) in the lateral pelvic region with H–E staining (**e**) and IHC for prostate-specific antigen (PSA) (**f**), showing the possibility of prostate adenocarcinoma origin. **g** Schematic illustration of the distribution of dissected lymph nodes. Lymph nodes metastatic from rectal adenocarcinoma and prostate adenocarcinoma are shown as *yellow* and *purple*, respectively

among different studies, and the immunoreactivity was classified into three grades: (+), which means all or most tumors are positive; (− +), which means some tumors are positive; and (−), which means most or all tumors are negative (Fig. 2). In parentheses, the positive rates for each of the markers are expressed as percentages, instead of (+), (− +), or (−) if the information was available (Fig. 2).

Retrieval of data from the Human Protein Atlas

The HPA [3], hosted by the Swedish Human Proteome Resource (HPR) program, is available at the web address: http://www.proteinatlas.org/index.php. This database provides the expression profiles at the protein levels, as well as IHC images for a wide variety of tissues and cell lines. The antibody information used for each of IHC can also be retrieved. This database yielded IHC data for colorectal adenocarcinoma, bladder adenocarcinoma, prostate adenocarcinoma, ovarian adenocarcinoma, cervical carcinoma, and endometrial adenocarcinoma; however, it does not classify ovarian carcinoma into histological subtypes. The immunoreactivity in the HPA database is classified into four grades: strong, moderate, weak, and negative (Supplementary Fig. 1). Figure 3 summarizes the positive rates of IHC markers retrieved from the HPA database in December, 2010.

Combining the results of the literature survey shown in Fig. 2 and those from the HPA database shown in Fig. 3 provides panels of IHC markers for the differential diagnosis of rectal adenocarcinoma from carcinomas of other pelvic organs (Table 2).

IHC markers for carcinomas of the pelvic organs

Figure 2 summarizes the findings of the 91 studies [4–94], including the results of IHC analyses and the information of the IHC markers.

Table 1 Genes analyzed for immunohistochemistry of pelvic carcinomas

Official symbol	Official full name	Other names	Chromosomal localization
KRT7	Keratin 7	K7; CK7; SCL; K2C7; MGC3625; MGC129731	12q12-q13
KRT20	Keratin 20	K20; CD20; CK20; KRT21; MGC35423	17q21.2
CEACAM5	Carcinoembryonic antigen-related cell adhesion molecule 5	CEA; CD66e; DKFZp781M2392	19q13.1-q13.2
CDX2	Caudal type homeobox 2	CDX3; CDX-3	13q12.3
VIL1	Villin 1	VIL; D2S1471	2q35-q36
TFF1	Trefoil factor 1	pS2; BCEI; HPS2; HP1.A; pNR-2; D21S21	21q22.3
THBD	Thrombomodulin	TM; THRM; CD141	20p11.2
UPK3A	Uroplakin 3A	UPK3; UPIII; UPIIIA; MGC119178	22q13.31
KLK3	Kallikrein-related peptidase 3	APS; PSA; hK3; KLK2A1	19q13.41
ACPP	Acid phosphatase, prostate	PAP; ACP3; ACP-3	3q21-q23
SLC45A3	Solute carrier family 45, member 3	PRST (prostein); IPCA6; IPCA-2; IPCA-6; IPCA-8; PCANAP2; PCANAP6; PCANAP8	1q32.1
AMACR	Alpha-methylacyl-CoA racemase	RACE; CBAS4 (P504S)	5p13
EPCAM	Epithelial cell adhesion molecule	EGP; ESA; KSA; M4S1; MK-1; EGP-2; EGP34; EGP40; KS1/4; MIC18; TROP1; CO-17A; Ep-CAM; hEGP-2; CO17-1A; GA733-2; TACST-1; TACSTD1	2p21
B3GAT1	Beta-1,3-glucuronyltransferase 1 (glucuronosyltransferase P)	NK1; CD57; HNK1; LEU7; NK-1; GLCATP; GLCUATP	11q25
NKX3-1	NK3 homeobox 1	NKX3; BAPX2; NKX3A; NKX3.1	8p21
MUC5AC	Mucin 5AC, oligomeric mucus/gel-forming	MUC5	11p15.5
WT1	Wilms tumor 1	GUD; WAGR; WT33; WIT-2	11p13
MUC16	Mucin 16, cell surface associated	CA125; FLJ14303	19p13.2
MSLN	Mesothelin	MPF; SMR; CAK1	16p13.3
CDKN2A	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	ARF; MLM; p14; p16; p19; CMM2; INK4; MTS1; TP16; CDK4I; CDKN2; INK4a; p14ARF; p16INK4; p16INK4a	9p21
VIM	Vimentin	FLJ36605	10p13
ESR1	Estrogen receptor 1	ER; ESR; Era; ESRA; NR3A1; DKFZp686N23123	6q25.1
PGR	Progesterone receptor	PR; NR3C3	11q22-q23
BCL2	B-cell CLL/lymphoma 2	Bcl-2	18q21.33

Keratins (KRTs)

Along with actins and tubulins, keratins (KRTs) are classified into the intermediate filament (IF) proteins, with KRTs subclassified from KRT1 to KRT20. KRT7 is a 54 kDa basic protein and KRT20 is a 46 kDa acidic protein, and most studies of keratins support the notion that KRT20 is positive in rectal adenocarcinoma, whereas KRT7 is negative. Carcinomas of other pelvic organs also show distinct patterns of immunoreactivity for KRT7 and KRT20, indicating that they are good markers for differential diagnosis.

CEACAM5, CDX2 and VIL1

In 1965, Gold and Freedman [95] discovered a tumor-specific antigen in human colorectal carcinoma, now known as carcinoembryonic antigen (CEA), using immunological tolerance and absorption techniques. CEA is a high molecular glycoprotein with 180 kDa, which is encoded by *carcinoembryonic antigen-related cell adhesion molecule 5* (*CEACAM5*) gene [96] and belongs to a family of membrane glycoproteins. CEA functions as a cell adhesion molecule and is important during embryogenesis and tumor development [97]. CEA is positive not only in rectal adenocarcinoma, but also in bladder adenocarcinoma and cervical carcinoma, whereas it is negative in ovarian mucinous and serous adenocarcinomas and endometrial adenocarcinoma.

In 1997, Drummond et al. [98] cloned the cDNA and amino acid sequences of the *Caudal type homeobox 2* (*CDX2*) gene from a human jejunal cDNA library. Bai et al. [99] also reported that CDX2 up-regulates the

	KRT7	KRT20	CEACAM5	CDX2	VIL1	TFF1 (pS2)	THBD	UPK3A	KLK3 (PSA)	ACPP (PAP)	AMACR	EPCAM	B3GAT1 (CD57)	NKX3-1	MUC5AC	WT1	MUC16 (CA125)	MSLN	CDKN2A (p16)	VIM	ESR1	PGR	BCL2
Rectal adenocarcinoma	87 (98%)	97 (100%)	76 (100%)	23 (100%)	11 (100%)	49 (85%)	92 (0%)	54 (-)	71 (0%)	87 (0%)	84 (70%)	94 (100%)	32 (-)		37 (33%)	55 (-)	87 (28%)	21 (+)	53 (-)	42 (-)	19 (-)	19 (-)	
	37 (33%)	90 (100%)	87 (97%)	90 (100%)	56 (100%)	14 (+)		57 (-)	87 (0%)	21 (-)	62 (45%)				44 (26%)	82 (-)	55 (-)	24 (+)		47 (-)	42 (-)		
	76 (4%)	76 (100%)	41 (+)	60 (100%)	84 (93%)				21 (-)		47 (32%)				6 (0%)								
	30 (0%)	92 (94%)	42 (+)	76 (100%)	65 (96%)																		
	92 (0%)	90 (93%)	53 (+)	84 (100%)	21 (+)																		
	19 (-)	87 (86%)		47 (95%)	26 (+)																		
	26 (-)	71 (90%)		90 (90%)	55 (+)																		
	42 (-)	19 (+)		71 (80%)	86 (+)																		
	53 (-)	26 (+)		55 (+)																			
	55 (-)	42 (+)		53 (+)																			
	82 (-)	53 (+)		76 (+)																			
	86 (-)	55 (+)		82 (+)																			
	90 (-)	82 (+)		68 (+)																			
		86 (+)																					
Bladder adenocarcinoma	12 (+)	87 (73%)	87 (97%)	84 (47%)	84 (86%)	40 (72%)	92 (59%)	54 (60%)	12 (0%)	54 (0%)	84 (65%)	58 (100%)	32 (-)	32 (-)	40 (46%)		87 (28%)	24 (-)	79 (+)	87 (0%)			
	27 (+)	76 (63%)	76 (83%)	76 (13%)	21 (-)	46 (39%)	54 (49%)	57 (+)	54 (0%)	87 (0%)		94 (38%)											
	70 (+)	12 (+)	70 (+)				32 (+)	72 (+)	87 (0%)	27 (-)													
	72 (+)	27 (+)					57 (+)		27 (-)	31 (-)													
	76 (83%)	70 (+)					67 (+)		31 (-)	32 (-)													
	87 (82%)	72 (+)					70 (+)		32 (-)														
							72 (+)		34 (-)														
Prostate adenocarcinoma	27 (12%)	97 (32%)	87 (14%)	33 (6%)	29 (-)	10 (-+)	54 (0%)	54 (0%)	15 (97%)	54 (95%)	38 (94%)	94 (99%)	27 (94%)	15 (95%)	44 (0%)	61 (+)	87 (5%)	21 (-)		87 (0%)	19 (-+)		
	87 (5%)	71 (10%)	27 (12%)	60 (1%)	56 (-)	14 (-)		57 (-)	27 (94%)	27 (94%)	62 (90%)	74 (+)	32 (+)	9 (+)	17 (-)	29 (-)	29 (-)	24 (-)					
	12 (-)	12 (-)	21 (-)	71 (0%)		49 (-)			87 (86%)	87 (82%)	5 (+)	32 (-+)		32 (+)									
				43 (-)					54 (85%)	21 (+)	31 (+)												
				92 (-)					71 (80%)	31 (+)	32 (+)												
									21 (+)	34 (+)	34 (+)												
									31 (+)	43 (+)	49 (+)												
									32 (+)		91 (+)												
									43 (+)														
Ovarian mucinous adenocarcinoma	30 (100%)	90 (74%)	19 (-)	90 (40%)	65 (15%)	49 (-)		54 (-)	20 (10%)		38 (7%)	94 (97%)			6 (100%)	35 (+)	20 (20%)	20 (30%)		55 (-+)	45 (86%)	45 (50%)	
	93 (100%)	97 (68%)	47 (-)	47 (22%)	55 (-)			57 (-)			47 (0%)				97 (88%)	4 (-)	42 (-+)				20 (40%)	19 (-+)	
	97 (98%)	20 (20%)	82 (-)	60 (20%)							62 (0%)				55 (+)	55 (-)	78 (-+)				19 (-+)	69 (-)	
	90 (74%)	93 (0%)		20 (20%)											75 (+)		55 (-)				89 (-)		
	20 (40%)	14 (+)		30 (0%)																			
	14 (+)	19 (+)		88 (-)																			
	19 (+)	41 (+)																					
	41 (+)	55 (+)																					
	42 (+)	75 (+)																					
	55 (+)	30 (-+)																					
	75 (+)																						
Ovarian serous adenocarcinoma	30 (100%)	20 (0%)	21 (-+)	20 (0%)	65 (15%)	14 (-+)	70 (-)	54 (-)	20 (0%)	21 (-)	38 (7%)	94 (97%)				29 (97%)	20 (89%)	20 (94%)	69 (-+)	55 (-+)	20 (83%)	19 (-+)	
	93 (100%)	93 (0%)	47 (-)	30 (0%)	11 (0%)	49 (-)		57 (-)	21 (-)		62 (0%)					64 (50%)	55 (+)	21 (+)			19 (-+)		
	20 (83%)	30 (-+)	70 (-)	47 (-)	21 (-)											4 (+)	75 (+)	24 (+)					
	19 (+)	19 (-)		60 (-)												19 (+)	42 (-+)						
	41 (+)	41 (-)		82 (-)												35 (+)							
	42 (+)	70 (-)		88 (-)												55 (+)							
	70 (+)	75 (-)														81 (+)							
	75 (+)	82 (-)														82 (+)							
	82 (+)																						
Cervical adenocarcinoma	13 (100%)	53 (-)	51 (96%)	85 (21%)	11 (+)	80 (-+)		54 (-)	87 (0%)	87 (0%)		94 (100%)					55 (-)		52 (96%)	51 (8%)	51 (38%)	50 (35%)	82 (-)
	87 (100%)	55 (-)	13 (62%)	53 (-+)				57 (-)											19 (+)	13 (7%)	50 (30%)	25 (27%)	
	90 (88%)		87 (50%)	76 (-)															22 (+)	87 (0%)	25 (20%)	28 (-+)	
	58 (+)		7 (+)																36 (+)	7 (-)	28 (-+)	89 (-+)	
	82 (+)		18 (+)																53 (+)	18 (-)	69 (-+)	19 (-)	
	55 (-)		19 (+)																55 (+)	19 (-)	7 (-)	55 (-)	
			53 (+)																63 (+)	55 (-)	19 (-)	83 (-)	
			55 (+)																77 (+)	82 (-)	55 (-)		
			82 (+)																78 (+)		83 (-)		
Endometrial adenocarcinoma	13 (99%)	13 (7%)	13 (27%)		56 (36%)	39 (-+)		54 (-)	87 (0%)	87 (0%)	62 (7%)	94 (94%)			44 (22%)	16 (+)	66 (+)	24 (-+)	8 (30%)	13 (97%)	51 (92%)	19 (+)	82 (+)
	87 (90%)	87 (5%)	87 (24%)		11 (17%)	49 (-)		57 (-)							59 (0.3%)	68 (-+)	82 (+)		55 (-)	51 (97%)	7 (+)	83 (+)	
	55 (+)	55 (-)	7 (-)														87 (86%)			7 (+)	19 (+)		
	75 (+)	73 (-)	18 (+)																	19 (+)	55 (+)		
	90 (+)	93 (-)	19 (-)																	19 (+)	83 (+)		
			55 (-)																	55 (+)			
			82 (-)																	82 (+)			

Fig. 2 Summary of the NCBI literature survey on immunohistochemical markers of pelvic carcinomas. Each of the 91 references is indicated by a leading number for each of the cells. The positive rates (*percentages*) for each of the markers are shown instead of (+), (− +), or (−), if the information was available

transcription of a tumor suppressor gene, *p21/WAF1/CIP1*. The disruption of CDX2 expression is considered to cause tumorigenesis of the gastrointestinal tract. Most reports of IHC on CDX2 were published after 2003 and document CDX2 positivity in almost all rectal adenocarcinomas, but negativity in prostate adenocarcinoma, ovarian serous adenocarcinoma, and cervical carcinoma.

Villin 1 (VIL1) is a calcium-dependently regulated, actin-binding protein of 95 kDa, and a major structural component of the brush border cytoskeleton [100, 101]. Bacchi and Gown [11] and Moll et al. [56] analyzed IHC expression of VIL1 in various tissues, and most studies suggest that VIL1 is positive in rectal adenocarcinoma. In 2003, Nishizuka et al. [65] comprehensively analyzed the expression profiles of colon adenocarcinoma and ovarian adenocarcinoma using cDNA microarrays and tissue arrays, and found that VIL1 and moesin are good markers for distinguishing these two diseases.

THBD and UPK3A

Thrombomodulin (THBD), which is a 120 kDa endothelial cell surface glycoprotein, and uroplakin 3A (UPK3A), which is a 47 kDa membrane glycoprotein, are commonly positive in bladder adenocarcinoma but negative in rectal adenocarcinoma.

KLK3, ACPP and prostein

Kallikrein-related peptidase 3 (KLK3), also known as "prostate-specific antigen (PSA)"; acid phosphatase prostate (ACPP), also known as "prostatic acid phosphatase (PAP)"; and solute carrier family 45, member 3 (SLC45A3), also known as "prostein", are selectively positive in prostate adenocarcinoma and negative in carcinomas of other pelvic organs, including rectal adenocarcinoma (Figs. 2, 3). KLK3 (PSA) belongs to the kallikrein family and is a 33 kDa single-chain glycoprotein

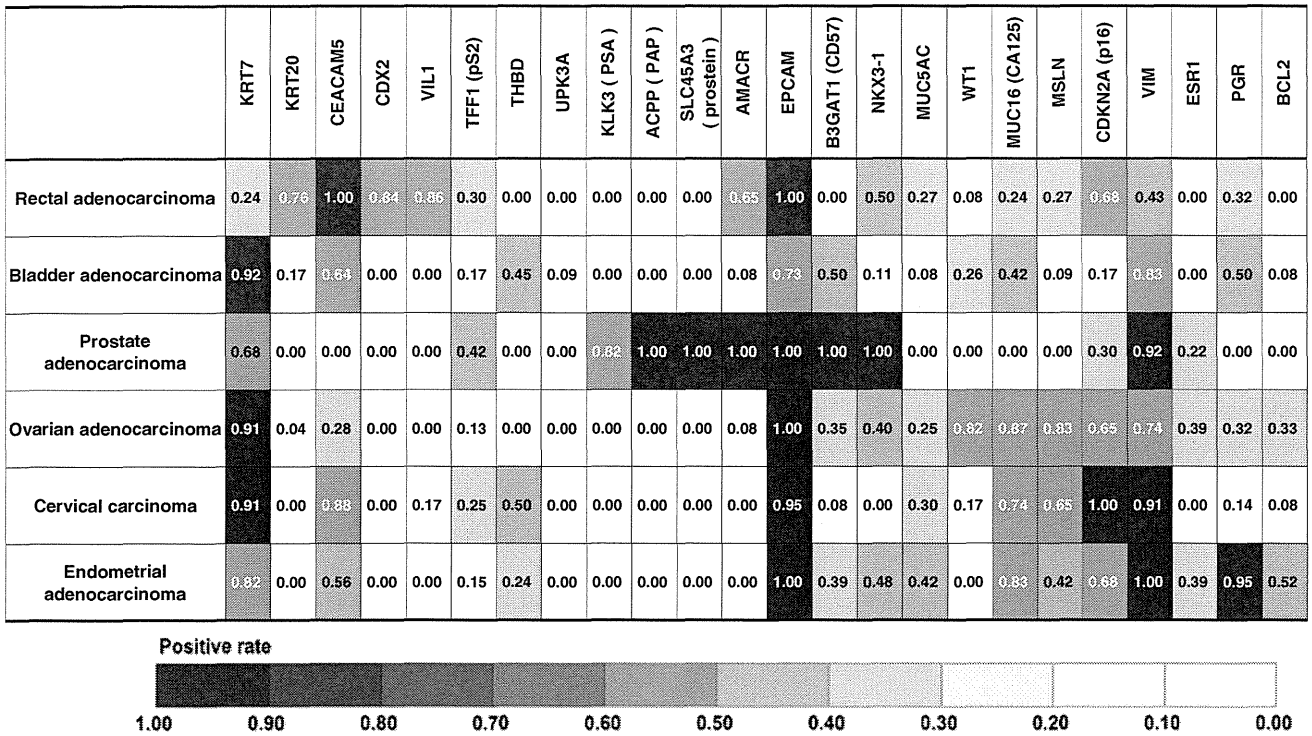

Fig. 3 Positive rates of immunoreactivity retrieved from the HPA

produced by the prostate epithelium and abundant in seminal fluid, which is both sensitive and specific in prostate adenocarcinoma [102, 103]. ACPP (PAP) is approximately 50 kDa and belongs to the kallikrein family encoding serine proteases [104], whereas SLC45A3 (prostein) is a prostate-specific transmembrane protein. Both are sensitive and specific in prostate adenocarcinoma, as is KLK3 (PSA).

MUC5AC

Mucins are a family of high molecular weight glycoproteins, expressed differentially in epithelia, depending on cell-type specificity; thus, some mucins can be used as IHC markers. Mucin 5AC, oligomeric mucus/gel-forming (MUC5AC), is positive in ovarian mucinous adenocarcinoma and negative in rectal adenocarcinoma.

WT1

Wilms' tumor (WT) develops in the kidney and is one of the most common solid tumors of childhood. The *WT1* gene and several other genes, including *WT2*, *WT3*, and *WT4*, have been associated with Wilms' tumors. In 2000, Shimizu et al. [81] immunohistochemically detected the WT1 protein in epithelial ovarian tumors and it is now considered a good marker of ovarian serous adenocarcinoma.

CDKN2A

Cyclin-dependent kinase inhibitor 2A (CDKN2A), also known as "p16", is selectively positive in cervical adenocarcinoma. In 1995, Stone et al. [105] demonstrated that the CDKN2A locus at 9p21 region codes two alternative transcripts from different promoters, p16 (INK4A) and p14 (ARF). Since then, homozygous deletions and genetic alterations of the *p16* gene have been reported in several malignancies, including melanoma and pancreatic carcinomas, indicating that p16 is a tumor suppressor [106, 107]. Lukas et al. [108] reported that p16 is a CDK inhibitor. IHC of p16 has also been reported in female genital tract cancers. Moreover, the detection of human papillomavirus (HPV) is a powerful strategy for the diagnosis of cervical carcinoma and squamous cell carcinoma, and it was also recently reported that cervical carcinoma can be diagnosed with its detection.

VIM, ESR1, and PGR

Vimentin (VIM), one of the main IF proteins expressed in mesenchymal cells [109], is positive in endometrial

Table 2 Proposal for sets of immunohistochemical markers for the differential diagnosis of rectal adenocarcinoma

Differential diagnosis	Immunohistochemical markers	
	Positive for rectal adenocarcinoma and negative for the other carcinoma	Negative for rectal adenocarcinoma and positive for the other carcinoma
Rectal adenocarcinoma versus bladder adenocarcinoma	CDX2, VIL1	KRT7, THBD, UPK3A
Rectal adenocarcinoma versus prostate adenocarcinoma	CDX2, VIL1, CEACAM5	KLK3 (PSA), ACPP (PAP), SLC45A3 (prostein)
Rectal adenocarcinoma versus ovarian mucinous adenocarcinoma	CEACAM5, VIL1, CDX2	KRT7, MUC5AC
Rectal adenocarcinoma versus ovarian serous adenocarcinoma	KRT20, CEACAM5, CDX2	KRT7, WT1
Rectal adenocarcinoma versus cervical carcinoma	KRT20, CDX2	KRT7, p16 + HPV detection
Rectal adenocarcinoma versus endometrial adenocarcinoma	KRT20, CEACAM5, VIL1	KRT7, VIM, ESR1, PGR

adenocarcinoma, while estrogen receptor 1 (ESR1) and progesterone receptor (PR) are positive in endometrial carcinoma. All of these markers are negative in rectal adenocarcinoma.

Most of the references for the IHC markers in Fig. 2 reported consistent results and provided important information for the differential diagnosis. It is important to note that much of the negative data on immunoreactivity may not have been reported and are thus not reflected in this summary. Supplementary Fig. 1 shows some of the IHC results retrieved from the HPA database in December 2010, along with the number of informative cases and the positive rates for each marker. The positive rates for each of the IHC markers from the HPA database are further summarized in Fig. 3. The results of the literature survey in Fig. 2 were highly concordant with those of the HPA database (Fig. 3).

The histological classification of ovarian tumors by the World Health Organization is widely accepted and categorizes ovarian tumors with regard to their derivation from coelomic surface epithelial cells, germ cells, and mesenchyme. Kaku et al. [110] reported that ovarian tumors exhibit a wide variety of histological features and McCluggage [111] revealed that the pathogenesis of ovarian carcinoma is largely unknown because of the lack of a tumor progression model. For these reasons, it is difficult to classify ovarian carcinomas into histological subtypes.

Panels of IHC markers for differential diagnosis

This study focuses on how IHC can be used in a clinical setting. We proposed sets of IHC markers to assist in the differential diagnosis of rectal adenocarcinoma from carcinomas of other pelvic organs, including the urinary bladder, prostate, ovary, and uterus. In the panels of IHC markers listed in Table 2, the symbol [+] attached to the names of the markers designates "positive" in rectal adenocarcinoma and "negative" in the other pelvic organ carcinomas, whereas [−] designates "negative" in rectal adenocarcinoma and "positive" in the other pelvic organ carcinomas. For example, the following panel of markers was proposed for the differential diagnosis of rectal adenocarcinoma from bladder adenocarcinoma: CDX2[+], VIL1[+], KRT7[−], THBD[−] and UPK3A[−].

Conclusions

The Swedish HPR program was established to allow for a systematic exploration of the human proteome using antibody-based proteomics [3]. The main objective of the resource center is to produce specific antibodies to human target proteins. These antibodies are used to study the expression profiles in cells and tissues and to perform functional analyses. The HPA version 1.1 was released in November 2005. In addition to the HPA, the NCBI (http://www.ncbi.nih.gov) and Ensembl databases (http://www.ensembl.org), other public databases on life sciences, including the Human Proteome Organization, the Universal Protein Resource, the Swiss-Prot protein knowledge base, and the Vega genome browser, are currently available. Much information has been accumulated on these public databases, which provide a valuable resource for clinicians and researchers. It is important not only to utilize their data, but also to register our novel data to promote further development of these databases. Finally,

it is important to establish the internationally standardized controls for those data, particularly for image data.

In this review, we proposed panels of IHC markers for the differential diagnosis of pelvic cancers. To utilize the information, it is important to remember that the expression of each of the markers can vary depending on the histological subtypes and differential status. Especially in poorly differentiated adenocarcinoma and signet-ring cell carcinoma, IHC staining of the markers is decreased [112] and the diagnostic biopsy material is limited [113]. In this regard, the differential diagnosis of undifferentiated adenocarcinoma is more difficult than that of differentiated adenocarcinoma. Furthermore, to use the information gained from this review, we must remember that IHC results can be influenced by many factors, namely, the fixation of materials, the preservation of materials, antibodies, IHC methods, and so on. Concerning differential diagnoses, clinical characteristics (such as sex, age, and history), macroscopic distribution of the tumor, and conventional histological findings are as important as IHC status. Considering all that has been discussed in this review, the panels of IHC markers for the differential diagnosis of pelvic carcinomas can be maximally utilized, not only by general surgeons, but also by urologists and gynecologists.

Conflict of interest Koh Miura and his co-authors have no conflict of interest.

References

1. Miura K, Kobayashi T, Funayama Y, Fukushima K, Ogawa H, Oyama A, et al. Giant T4 rectal carcinoma mimicking urinary bladder adenocarcinoma accurately diagnosed by immunohistochemistry and successfully treated with total pelvic exenteration: report of a case. Surg Today. 2008;38:261–5.
2. Giuliani A, Galati G, Demoro M, Scimò M, Ciardi A, Basso L. Endoluminal metastasis of colon cancer to the urinary bladder via the ureter: report of a case. Surg Today. 2010;40:1093–6.
3. Uhlén M, Björling E, Agaton C, Szigyarto CA, Amini B, Andersen E, et al. A human protein atlas for normal and cancer tissues based on antibody proteomics. Mol Cell Proteomics. 2005;4:1920–32.
4. Acs G, Pasha T, Zhang PJ. WT1 is differentially expressed in serous, endometrioid, clear cell, and mucinous carcinomas of the peritoneum, fallopian tube, ovary, and endometrium. Int J Gynecol Pathol. 2004;23:110–8.
5. Adley BP, Yang XJ. Application of alpha-methylacyl coenzyme A racemase immunohistochemistry in the diagnosis of prostate cancer: a review. Anal Quant Cytol Histol. 2006;28:1–13.
6. Albarracin CT, Jafri J, Montag AG, Hart J, Kuan SF. Differential expression of MUC2 and MUC5AC mucin genes in primary ovarian and metastatic colonic carcinoma. Hum Pathol. 2000;31:672–7.
7. Alkushi A, Irving J, Hsu F, Dupuis B, Liu CL, Rijn M, et al. Immunoprofile of cervical and endometrial adenocarcinomas using a tissue microarray. Virchows Arch. 2003;442:271–7.
8. Ansari-Lari MA, Staebler A, Zaino RJ, Shah KV, Ronnett BM. Distinction of endocervical and endometrial adenocarcinomas: immunohistochemical p16 expression correlated with human papillomavirus (HPV) DNA detection. Am J Surg Pathol. 2004;28:160–7.
9. Aslan G, Irer B, Tuna B, Yorukoglu K, Saatcioglu F, Celebi I. Analysis of NKX3.1 expression in prostate cancer tissues and correlation with clinicopathologic features. Pathol Res Pract. 2006;202:93–8.
10. Ather MH, Abbas F, Faruqui N, Israr M, Pervez S. Expression of pS2 in prostate cancer correlates with grade and Chromogranin A expression but not with stage. BMC Urol. 2004;4:14.
11. Bacchi CE, Gown AM. Distribution and pattern of expression of villin, a gastrointestinal-associated cytoskeletal protein, in human carcinomas: a study employing paraffin-embedded tissue. Lab Invest. 1991;64:418–24.
12. Bassily NH, Vallorosi CJ, Akdas G, Montie JE, Rubin MA. Coordinate expression of cytokeratins 7 and 20 in prostate adenocarcinoma and bladder urothelial carcinoma. Am J Clin Pathol. 2000;113:383–8.
13. Castrillon DH, Lee KR, Nucci MR. Distinction between endometrial and endocervical adenocarcinoma: an immunohistochemical study. Int J Gynecol Pathol. 2002;21:4–10.
14. Chou YY, Jeng YM, Kao HL, Chen T, Mao TL, Lin MC. Differentiation of ovarian mucinous carcinoma and metastatic colorectal adenocarcinoma by immunostaining with beta-catenin. Histopathology. 2003;43:151–6.
15. Chuang AY, DeMarzo AM, Veltri RW, Sharma RB, Bieberich CJ, Epstein JI. Immunohistochemical differentiation of high-grade prostate carcinoma from urothelial carcinoma. Am J Surg Pathol. 2007;31:1246–55.
16. Coosemans A, Moerman P, Verbist G, Maes W, Neven P, Vergote I, et al. Wilms' tumor gene 1 (WT1) in endometrial carcinoma. Gynecol Oncol. 2008;111:502–8.
17. Cozzi PJ, Wang J, Delprado W, Perkins AC, Allen BJ, Russell PJ, et al. MUC1, MUC2, MUC4, MUC5AC and MUC6 expression in the progression of prostate cancer. Clin Exp Metastasis. 2005;22:565–73.
18. Dabbs DJ, Geisinger KR, Norris HT. Intermediate filaments in endometrial and endocervical carcinomas. The diagnostic utility of vimentin patterns. Am J Surg Pathol. 1986;10:568–76.
19. Deavers MT, Malpica A, Silva EG. Immunohistochemistry in gynecological pathology. Int J Gynecol Cancer. 2003;13:567–79.
20. Dennis JL, Hvidsten TR, Wit EC, Komorowski J, Bell AK, Downie I, et al. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. Clin Cancer Res. 2005;11:3766–72.
21. Dennis JL, Oien KA. Hunting the primary: novel strategies for defining the origin of tumours. J Pathol. 2005;205:236–47.
22. El-Ghobashy AA, Shaaban AM, Innes J, Prime W, Herrington CS. Differential expression of cyclin-dependent kinase inhibitors and apoptosis-related proteins in endocervical lesions. Eur J Cancer. 2007;43:2011–8.
23. Fraggetta F, Pelosi G, Cafici A, Scollo P, Nuciforo P, Viale G. CDX2 immunoreactivity in primary and metastatic ovarian mucinous tumours. Virchows Arch. 2003;443:782–6.
24. Frierson HF Jr, Moskaluk CA, Powell SM, Zhang H, Cerilli LA, Stoler MH, et al. Large-scale molecular and tissue microarray analysis of mesothelin expression in common human carcinomas. Hum Pathol. 2003;34:605–9.
25. Fujiwara H, Tortolero-Luna G, Mitchell MF, Koulos JP, Wright TC Jr. Adenocarcinoma of the cervix. Expression and clinical significance of estrogen and progesterone receptors. Cancer. 1997;79:505–12.
26. Geller SA, Dhall D, Alsabeh R. Application of immunohistochemistry to liver and gastrointestinal neoplasms: liver,

stomach, colon, and pancreas. Arch Pathol Lab Med. 2008;132:490–9.
27. Genega EM, Hutchinson B, Reuter VE, Gaudin PB. Immunophenotype of high-grade prostatic adenocarcinoma and urothelial carcinoma. Mod Pathol. 2000;13:1186–91.
28. Ghandour FA, Attanoos R, Nahar K, Gee JW, Bigrigg A, Ismail SM. Immunocytochemical localization of oestrogen and progesterone receptors in primary adenocarcinoma of the cervix. Histopathology. 1994;24:49–55.
29. Goldstein NS. Immunophenotypic characterization of 225 prostate adenocarcinomas with intermediate or high Gleason scores. Am J Clin Pathol. 2002;117:471–7.
30. Groisman GM, Meir A, Sabo E. The value of Cdx2 immunostaining in differentiating primary ovarian carcinomas from colonic carcinomas metastatic to the ovaries. Int J Gynecol Pathol. 2004;23:52–7.
31. Hameed O, Humphrey PA. Immunohistochemistry in diagnostic surgical pathology of the prostate. Semin Diagn Pathol. 2005;22:88–104.
32. Hammerich KH, Ayala GE, Wheeler TM. Application of immunohistochemistry to the genitourinary system (prostate, urinary bladder, testis, and kidney). Arch Pathol Lab Med. 2008;132:432–40.
33. Herawi M, De Marzo AM, Kristiansen G, Epstein JI. Expression of CDX2 in benign tissue and adenocarcinoma of the prostate. Hum Pathol. 2007;38:72–8.
34. Humphrey PA. Diagnosis of adenocarcinoma in prostate needle biopsy tissue. J Clin Pathol. 2007;60:35–42.
35. Hylander B, Repasky E, Shrikant P, Intengan M, Beck A, Driscoll D, et al. Expression of Wilms tumor gene (WT1) in epithelial ovarian cancer. Gynecol Oncol. 2006;101:12–7.
36. Ishikawa M, Fujii T, Masumoto N, Saito M, Mukai M, Nindl I, et al. Correlation of p16INK4A overexpression with human papillomavirus infection in cervical adenocarcinomas. Int J Gynecol Pathol. 2003;22:378–85.
37. Ji H, Isacson C, Seidman JD, Kurman RJ, Ronnett BM. Cytokeratins 7 and 20, Dpc4, and MUC5AC in the distinction of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors: Dpc4 assists in identifying metastatic pancreatic carcinomas. Int J Gynecol Pathol. 2002;21:391–400.
38. Jiang Z, Fanger GR, Woda BA, Banner BF, Algate P, Dresser K, et al. Expression of alpha-methylacyl-CoA racemase (P504 s) in various malignant neoplasms and normal tissues: astudy of 761 cases. Hum Pathol. 2003;34:792–6.
39. Koshiyama M, Yoshida M, Konishi M, Takemura M, Yura Y, Matsushita K, et al. Expression of pS2 protein in endometrial carcinomas: correlation with clinicopathologic features and sex steroid receptor status. Int J Cancer. 1997;74:237–44.
40. Kunze E, Krassenkova I, Fayyazi A. Tumor-associated neoexpression of the pS2 peptide and MUC5AC mucin in primary adenocarcinomas and signet ring cell carcinomas of the urinary bladder. Histol Histopathol. 2008;23:539–48.
41. Lagendijk JH, Mullink H, Van Diest PJ, Meijer GA, Meijer CJ. Tracing the origin of adenocarcinomas with unknown primary using immunohistochemistry: differential diagnosis between colonic and ovarian carcinomas as primary sites. Hum Pathol. 1998;29:491–7.
42. Lagendijk JH, Mullink H, van Diest PJ, Meijer GA, Meijer CJ. Immunohistochemical differentiation between primary adenocarcinomas of the ovary and ovarian metastases of colonic and breast origin. Comparison between a statistical and an intuitive approach. J Clin Pathol. 1999;52:283–90.
43. Lane Z, Epstein JI, Ayub S, Netto GJ. Prostatic adenocarcinoma in colorectal biopsy: clinical and pathologic features. Hum Pathol. 2008;39:543–9.
44. Lau SK, Weiss LM, Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. Am J Clin Pathol. 2004;122:61–9.
45. Lee BH, Hecht JL, Pinkus JL, Pinkus GS. WT1, estrogen receptor, and progesterone receptor as markers for breast or ovarian primary sites in metastatic adenocarcinoma to body fluids. Am J Clin Pathol. 2002;117:745–50.
46. Lipponen PK, Eskelinen MJ. Expression of pS2 protein in transitional cell bladder tumours. J Pathol. 1994;173:327–32.
47. Logani S, Oliva E, Arnell PM, Amin MB, Young RH. Use of novel immunohistochemical markers expressed in colonic adenocarcinoma to distinguish primary ovarian tumors from metastatic colorectal carcinoma. Mod Pathol. 2005;18:19–25.
48. Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, et al. Alpha-methylacyl-CoA racemase: a new molecular marker for prostate cancer. Cancer Res. 2002;62:2220–6.
49. Luqmani YA, Ryall G, Shousha S, Coombes RC. An immunohistochemical survey of pS2 expression in human epithelial cancers. Int J Cancer. 1992;50:302–4.
50. Masood S, Rhatigan RM, Wilkinson EW, Barwick KW, Wilson WJ. Expression and prognostic significance of estrogen and progesterone receptors in adenocarcinoma of the uterine cervix. An immunocytochemical study. Cancer. 1993;72:511–8.
51. McCluggage WG, Sumathi VP, McBride HA, Patterson A. A panel of immunohistochemical stains, including carcinoembryonic antigen, vimentin, and estrogen receptor, aids the distinction between primary endometrial and endocervical adenocarcinomas. Int J Gynecol Pathol. 2002;21:11–5.
52. McCluggage WG, Jenkins D. p16 immunoreactivity may assist in the distinction between endometrial and endocervical adenocarcinoma. Int J Gynecol Pathol. 2003;22:231–5.
53. McCluggage WG, Shah R, Connolly LE, McBride HA. Intestinal-type cervical adenocarcinoma in situ and adenocarcinoma exhibit a partial enteric immunophenotype with consistent expression of CDX2. Int J Gynecol Pathol. 2008;27:92–100.
54. Mhawech P, Uchida T, Pelte MF. Immunohistochemical profile of high-grade urothelial bladder carcinoma and prostate adenocarcinoma. Hum Pathol. 2002;33:1136–40.
55. Mittal K, Soslow R, McCluggage WG. Application of immunohistochemistry to gynecologic pathology. Arch Pathol Lab Med. 2008;132:402–23.
56. Moll R, Robine S, Dudouet B, Louvard D. Villin: a cytoskeletal protein and a differentiation marker expressed in some human adenocarcinomas. Virchows Arch B Cell Pathol Incl Mol Pathol. 1987;54:155–69.
57. Moll R, Wu XR, Lin JH, Sun TT. Uroplakins, specific membrane proteins of urothelial umbrella cells, as histological markers of metastatic transitional cell carcinomas. Am J Pathol. 1995;147:1383–97.
58. Momburg F, Moldenhauer G, Hämmerling GJ, Möller P. Immunohistochemical study of the expression of a Mr 34,000 human epithelium-specific surface glycoprotein in normal and malignant tissues. Cancer Res. 1987;47:2883–91.
59. Morrison C, Merati K, Marsh WL Jr, De Lott L, Cohn DE, Young G, et al. The mucin expression profile of endometrial carcinoma and correlation with clinical-pathologic parameters. Appl Immunohistochem Mol Morphol. 2007;15:426–31.
60. Moskaluk CA, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF Jr. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. Mod Pathol. 2003;16:913–9.
61. Nakatsuka S, Oji Y, Horiuchi T, Kanda T, Kitagawa M, Takeuchi T, et al. Immunohistochemical detection of WT1 protein in a variety of cancer cells. Mod Pathol. 2006;19:804–14.

62. Nassar A, Amin MB, Sexton DG, Cohen C. Utility of alpha-methylacyl coenzyme A racemase (p504 s antibody) as a diagnostic immunohistochemical marker for cancer. Appl Immunohistochem Mol Morphol. 2005;13:252–5.
63. Negri G, Egarter-Vigl E, Kasal A, Romano F, Haitel A, Mian C. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immunocytochemical correlations. Am J Surg Pathol. 2003;27:187–93.
64. Netinatsunthorn W, Hanprasertpong J, Dechsukhum C, Leetanaporn R, Geater A. WT1 gene expression as a prognostic marker in advanced serous epithelial ovarian carcinoma: an immunohistochemical study. BMC Cancer. 2006;6:90.
65. Nishizuka S, Chen ST, Gwadry FG, Alexander J, Major SM, Scherf U, et al. Diagnostic markers that distinguish colon and ovarian adenocarcinomas: identification by genomic, proteomic, and tissue array profiling. Cancer Res. 2003;63:5243–50.
66. Nur S, Chuang L, Ramaswamy G. Immunohistochemical characterization of cancer antigen in uterine cancers. Int J Gynecol Cancer. 2006;16:1903–10.
67. Obama H, Obama K, Takemoto M, Soejima Y, Shirahama T, Ohi Y, et al. Expression of thrombomodulin in the epithelium of the urinary bladder: a possible source of urinary thrombomodulin. Anticancer Res. 1999;19:1143–7.
68. Ohno S, Dohi S, Ohno Y, Kyo S, Sugiyama H, Suzuki N, et al. Immunohistochemical detection of WT1 protein in endometrial cancer. Anticancer Res. 2009;29:1691–5.
69. O'Neill CJ, McBride HA, Connolly LE, Deavers MT, Malpica A, McCluggage WG. High-grade ovarian serous carcinoma exhibits significantly higher p16 expression than low-grade serous carcinoma and serous borderline tumour. Histopathology. 2007;50:773–9.
70. Ordóñez NG. Thrombomodulin expression in transitional cell carcinoma. Am J Clin Pathol. 1998;110:385–90.
71. Owens CL, Epstein JI, Netto GJ. Distinguishing prostatic from colorectal adenocarcinoma on biopsy samples: the role of morphology and immunohistochemistry. Arch Pathol Lab Med. 2007;131:599–603.
72. Parker DC, Folpe AL, Bell J, Oliva E, Young RH, Cohen C, et al. Potential utility of uroplakin III, thrombomodulin, high molecular weight cytokeratin, and cytokeratin 20 in noninvasive, invasive, and metastatic urothelial (transitional cell) carcinomas. Am J Surg Pathol. 2003;27:1–10.
73. Pavlidis N, Fizazi K. Cancer of unknown primary (CUP). Crit Rev Oncol Hematol. 2005;54:243–50.
74. Poczatek RB, Myers RB, Manne U, Oelschlager DK, Weiss HL, Bostwick DG, et al. Ep-Cam levels in prostatic adenocarcinoma and prostatic intraepithelial neoplasia. J Urol. 1999;162:1462–6.
75. Prat J. Ovarian carcinomas, including secondary tumors: diagnostically challenging areas. Mod Pathol. 2005;18(Suppl 2):S99–111.
76. Raspollini MR, Nesi G, Baroni G, Girardi LR, Taddei GL. Immunohistochemistry in the differential diagnosis between primary and secondary intestinal adenocarcinoma of the urinary bladder. Appl Immunohistochem Mol Morphol. 2005;13:358–62.
77. Riethdorf L, O'Connell JT, Riethdorf S, Cviko A, Crum CP. Differential expression of MUC2 and MUC5AC in benign and malignant glandular lesions of the cervix uteri. Virchows Arch. 2000;437:365–71.
78. Rocha AS, Bozzetti MC, Kirschnick LS, Edelweiss MI. Antibody anti-p16(INK4a) in cervical cytology. Acta Cytol. 2009;53:253–62.
79. Rosenblatt R, Jonmarker S, Lewensohn R, Egevad L, Sherif A, Kälkner KM, et al. Current status of prognostic immunohistochemical markers for urothelial bladder cancer. Tumour Biol. 2008;29:311–22.
80. Saegusa M, Hashimura M, Hara A, Okayasu I. Up-regulation of pS2 expression during the development of adenocarcinomas but not squamous cell carcinomas of the uterine cervix, independently of expression of c-jun or oestrogen and progesterone receptors. J Pathol. 2000;190:554–63.
81. Shimizu M, Toki T, Takagi Y, Konishi I, Fujii S. Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. Int J Gynecol Pathol. 2000;19:158–63.
82. Shutter J, Atkins KA, Ghartey K, Herzog TJ. Clinical applications of immunohistochemistry in gynecological malignancies. Int J Gynecol Cancer. 2007;17:311–5.
83. Staebler A, Sherman ME, Zaino RJ, Ronnett BM. Hormone receptor immunohistochemistry and human papillomavirus in situ hybridization are useful for distinguishing endocervical and endometrial adenocarcinomas. Am J Surg Pathol. 2002;26:998–1006.
84. Suh N, Yang XJ, Tretiakova MS, Humphrey PA, Wang HL. Value of CDX2, villin, and alpha-methylacyl coenzyme A racemase immunostains in the distinction between primary adenocarcinoma of the bladder and secondary colorectal adenocarcinoma. Mod Pathol. 2005;18:1217–22.
85. Sullivan LM, Smolkin ME, Frierson HF Jr, Galgano MT. Comprehensive evaluation of CDX2 in invasive cervical adenocarcinomas: immunopositivity in the absence of overt colorectal morphology. Am J Surg Pathol. 2008;32:1608–12.
86. Tamboli P, Mohsin SK, Hailemariam S, Amin MB. Colonic adenocarcinoma metastatic to the urinary tract versus primary tumors of the urinary tract with glandular differentiation: a report of 7 cases and investigation using a limited immunohistochemical panel. Arch Pathol Lab Med. 2002;126:1057–63.
87. Torenbeek R, Lagendijk JH, Van Diest PJ, Bril H, van de Molengraft FJ, Meijer CJ. Value of a panel of antibodies to identify the primary origin of adenocarcinomas presenting as bladder carcinoma. Histopathology. 1998;32:20–7.
88. Tornillo L, Moch H, Diener PA, Lugli A, Singer G. CDX-2 immunostaining in primary and secondary ovarian carcinomas. J Clin Pathol. 2004;57:641–3.
89. Vang R, Gown AM, Barry TS, Wheeler DT, Ronnett BM. Immunohistochemistry for estrogen and progesterone receptors in the distinction of primary and metastatic mucinous tumors in the ovary: an analysis of 124 cases. Mod Pathol. 2006;19:97–105.
90. Vang R, Gown AM, Wu LS, Barry TS, Wheeler DT, Yemelyanova A, et al. Immunohistochemical expression of CDX2 in primary ovarian mucinous tumors and metastatic mucinous carcinomas involving the ovary: comparison with CK20 and correlation with coordinate expression of CK7. Mod Pathol. 2006;19:1421–8.
91. Varma M, Jasani B. Diagnostic utility of immunohistochemistry in morphologically difficult prostate cancer: review of current literature. Histopathology. 2005;47:1–16.
92. Wang HL, Lu DW, Yerian LM, Alsikafi N, Steinberg G, Hart J, et al. Immunohistochemical distinction between primary adenocarcinoma of the bladder and secondary colorectal adenocarcinoma. Am J Surg Pathol. 2001;25:1380–7.
93. Wauters CC, Smedts F, Gerrits LG, Bosman FT, Ramaekers FC. Keratins 7 and 20 as diagnostic markers of carcinomas metastatic to the ovary. Hum Pathol. 1995;26:852–5.
94. Went PT, Lugli A, Meier S, Bundi M, Mirlacher M, Sauter G, et al. Frequent EpCam protein expression in human carcinomas. Hum Pathol. 2004;35:122–8.
95. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med. 1965;121:439–62.
96. Schrewe H, Thompson J, Bona M, Hefta LJ, Maruya A, Hassauer M, et al. Cloning of the complete gene for carcinoembryonic antigen: analysis of its promoter indicates a region conveying cell type-specific expression. Mol Cell Biol. 1990;10:2738–48.