Cancer-Associated Perturbations in Alternative Pre-messenger RNA Splicing

Lulzim Shkreta, Brendan Bell, Timothée Revil, Julian P. Venables, Panagiotis Prinos, Sherif Abou Elela and Benoit Chabot

Abstract

For most of our 25,000 genes, the removal of introns by pre-messenger RNA (pre-mRNA) splicing represents an essential step toward the production of functional messenger RNAs (mRNAs). Alternative splicing of a single pre-mRNA results in the production of different mRNAs. Although complex organisms use alternative splicing to expand protein function and phenotypic diversity, patterns of alternative splicing are often altered in cancer cells. Alternative splicing contributes to tumorigenesis by producing splice isoforms that can stimulate cell proliferation and cell migration or induce resistance to apoptosis and anticancer agents. Cancer-specific changes in splicing profiles can occur through mutations that are affecting splice sites and splicing

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control elements, and also by alterations in the expression of proteins that control splicing decisions. Recent progress in global approaches that interrogate splicing diversity should help to obtain specific splicing signatures for cancer types. The development of innovative approaches for annotating and reprogramming splicing events will more fully establish the essential contribution of alternative splicing to the biology of cancer and will hopefully provide novel targets and anticancer strategies. Metazoan genes are usually made up of several exons interrupted by introns. The introns are removed from the pre-mRNA by RNA splicing. In conjunction with other maturation steps, such as capping and polyadenylation, the spliced mRNA is then transported to the cytoplasm to be translated into a functional protein. The basic mechanism of splicing requires accurate recognition of each extremity of each intron by the spliceosome. Introns are identified by the binding of U1 snRNP to the 5′ splice site and the U2AF65/U2AF35 complex to the 3′ splice site. Following these interactions, other proteins and snRNPs are recruited to generate the complete spliceosomal complex needed to excise the intron. While many introns are constitutively removed by the spliceosome, other splice junctions are not used systematically, generating the phenomenon of alternative splicing. Alternative splicing is therefore the process by which a single species of pre-mRNA can be matured to produce different mRNA molecules (Fig. 1). Depending on the number and types of alternative splicing events, a pre-mRNA can generate from two to several thousands different mRNAs leading to the production of a corresponding number of proteins. It is now believed that the expression of at least 70 % of human genes is subjected to alternative splicing, implying an enormous contribution to proteomic diversity, and by extension, to the development and the evolution of complex animals. Defects in splicing have been associated with human diseases (Caceres and Kornblihtt, Trends Genet 18(4):186–93, 2002, Cartegni et al., Nat Rev Genet 3(4):285–98, 2002, Pagani and Baralle, Nat Rev Genet 5(5):389–96, 2004), including cancer (Brinkman, Clin Biochem 37(7):584–94, 2004, Venables, Bioessays 28(4):378–86, 2006, Srebrow and Kornblihtt, J Cell Sci 119(Pt 13):2635–2641, 2006, Revil et al., Bull Cancer 93(9):909–919, 2006, Venables, Transworld Res Network, 2006, Pajares et al., Lancet Oncol 8(4):349–57, 2007, Skotheim and Nees, Int J Biochem Cell Biol 39:1432–1449, 2007). Numerous studies have now confirmed the existence of specific differences in the alternative splicing profiles between normal and cancer tissues. Although there are a few cases where specific mutations are the primary cause for these changes, global alterations in alternative splicing in cancer cells may be primarily derived from changes in the expression of RNA-binding proteins that control splice site selection. Overall, these cancer-specific differences in alternative splicing offer an immense potential to improve the diagnosis and the prognosis of cancer. This review will focus on the functional impact of cancer-associated alternative splicing variants, the molecular determinants that alter the splicing decisions in cancer cells, and future therapeutic strategies.

Contents

1   Introduction

For most of our 25,000 genes, the removal of introns by pre-messenger RNA (pre-mRNA) splicing represents an essential step toward the production of functional messenger RNAs (mRNA). Alternative splicing of a single pre-mRNA results in the production of different mRNAs. Although complex organisms use alternative splicing to expand protein function and phenotypic diversity, patterns of alternative splicing are often altered in cancer cells. Alternative splicing contributes to tumorigenesis by producing splice isoforms that can stimulate cell proliferation and cell migration or induce resistance to apoptosis and anticancer agents. Cancer-specific changes in splicing profiles can occur through mutations that are affecting splice sites and splicing control elements, and also by alterations in the expression of proteins that control splicing decisions. Recent progress in global approaches that interrogate splicing diversity should help to obtain specific splicing signatures for cancer types. The development of innovative approaches for annotating and reprogramming splicing events will more fully establish the essential contribution of alternative splicing to the biology of cancer and will hopefully provide novel targets and anticancer strategies.

Metazoan genes are usually made up of several exons interrupted by introns. The introns are removed from the pre-mRNA by RNA splicing. In conjunction with other maturation steps, such as capping and polyadenylation, the spliced mRNA is then transported to the cytoplasm to be translated into a functional protein. The basic mechanism of splicing requires accurate recognition of each extremity of each intron by the spliceosome. Introns are identified by the binding of U1 snRNP to the 5′ splice site and the U2AF65/U2AF35 complex to the 3′ splice site. Following these interactions, other proteins and snRNPs are recruited to generate the complete spliceosomal complex needed to excise the intron.

While many introns are constitutively removed by the spliceosome, other splice junctions are not used systematically, generating the phenomenon of alternative splicing. Alternative splicing is therefore the process by which a single species of pre-mRNA can be matured to produce different mRNA molecules (Fig. 1). Depending on the number and types of alternative splicing events, a pre-mRNA can generate from two to several thousands different mRNAs leading to the production of a corresponding number of proteins. It is now believed that the expression of at least 70 % of human genes is subjected to alternative splicing, implying an enormous contribution to proteomic diversity, and by extension, to the development and the evolution of complex animals.

Defects in splicing have been associated with human diseases [1–3], including cancer [4–10]. Numerous studies have now confirmed the existence of specific differences in the alternative splicing profiles between normal and cancer tissues. Although there are a few cases where specific mutations are the primary cause for these changes, global alterations in alternative splicing in cancer cells may be primarily derived from changes in the expression of RNA-binding proteins that control splice site selection. Overall, these cancer-specific differences in alternative splicing offer an immense potential to improve the diagnosis and the prognosis of cancer.

This review will focus on the functional impact of cancer-associated alternative splicing variants, the molecular determinants that alter the splicing decisions in cancer cells, and future therapeutic strategies.

2   Function of Cancer-Associated Splice Variants

Alternative splicing is part of the normal expression program of the majority of human genes. The initial reports describing the importance of alternative splicing in the control of sex determination in *Drosophila* [11] were followed by several examples linking splicing to the regulation of gene expression in human cells. For example, it was shown that alternative splicing controls the production of membrane-associated or secreted forms of immunoglobulins [12], and the synthesis of hormones with distinct physiological functions [13]. The most striking examples of the effect of splicing on gene expression are found in the nervous system where alternative splicing is used to expand the functional repertoire of receptor molecules. One notable example is the alternative splicing of the Slo gene that leads to the production of different proteins playing a role in the perception of different sound frequencies during audition [14].

The contribution of alternative splicing to gene expression is not always to expand protein diversity. Indeed, alternative splicing can also regulate the level of gene expression by producing mRNA isoforms containing premature stop codons that activate nonsense-mediated RNA decay (NMD). However, a global analysis

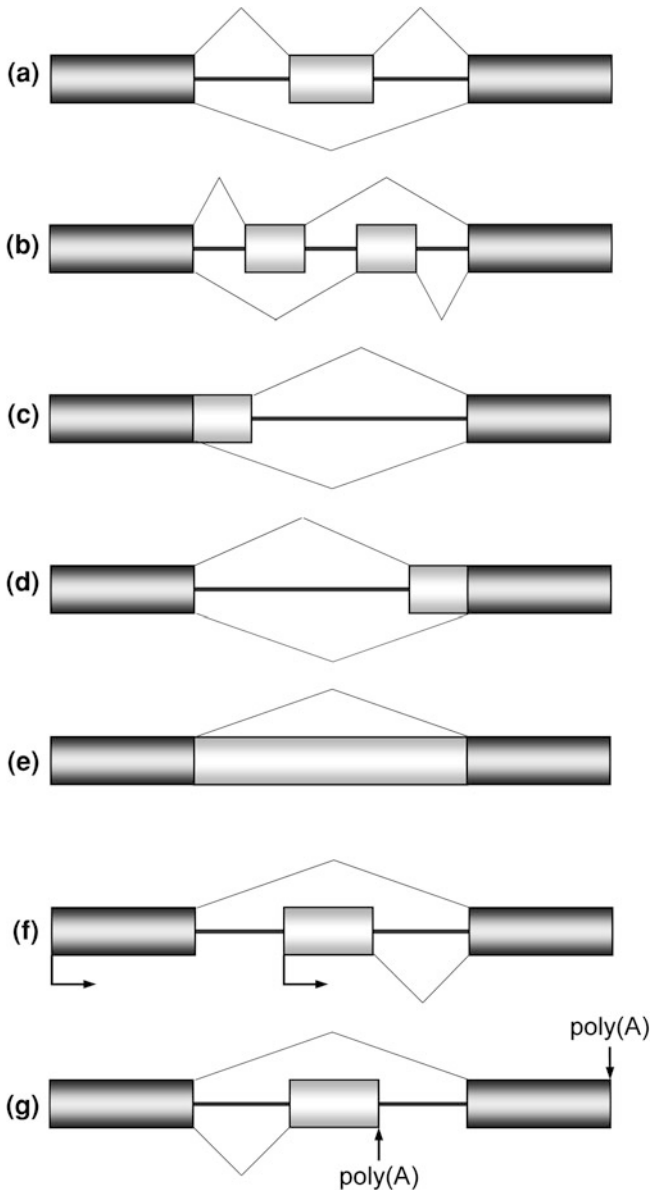**Fig. 1** Patterns of alternative splicing. *Gray* boxes represent exons or exonic fragments that are alternatively spliced. **a** cassette exon, **b** mutually exclusive exons, **c** alternative 5′ splice sites, **d** alternative 3′ splice sites, **e** intron retention, **f** alternative promoters can affect the identity of the first exon, and **g** alternative polyadenylation sites can impact the structure of the terminal exon

of the effect of knocking-down a specific NMD component on the abundance of 3126 alternative splicing events suggests that this pathway is not general but rather affects a selected group of pre-mRNAs [15]. Interestingly, splicing factors such as PTB and SC35 autoregulate their own expression by promoting the production of the NMD-sensitive isoforms [16–18]. Recent studies indicate that this mode of regulation is a hallmark of many splicing regulatory factors that belong to the SR and hnRNP family of proteins [17, 19].

Despite the involvement of alternative splicing in the expression and diversity of selective sets of genes, the global impact of the functional diversity imparted by alternative splicing still remains incomplete and controversial [20]. However, some of the best available evidence documenting the breadth and significance of alternative splicing has been provided by the study of cancer cells. Selected examples ordered by cellular functions are presented below.

2.1 Cellular Proliferation

Expression of the fibroblast growth factor receptor (FGFR) family is closely linked to cellular proliferation and cancer. Alternative splicing of FGFR1 and FGFR2 pre-mRNAs produce splice variants that have different affinities for their respective ligands. The FGFR2-IIIc splice isoforms is overexpressed in advanced stages of prostate cancers and transforms human mammary epithelial cells when expressed ectopically [21, 22]. FGFR2-IIIc accumulates in mesenchymal cells while FGFR2-IIIb is preferentially produced in epithelial cells. In a rat model system, prostate cancer cells expressing the FGFR2-IIIc-specific exon in a reporter construct revealed unexpected mesenchymal-epithelial transitions in primary tumors and lung micrometastases, revealing their phenotypic plasticity [23].

Proto-oncogenes and tumor-suppressor genes are essential players in the regulation of cellular proliferation. The majority of these genes express isoforms that are generated by alternative splicing. For example, the pre-mRNA encoding the p53 protein is alternatively spliced to produce isoforms whose abundance vary in breast tumors [24]. Other members of the p53 family such as p73 and p63 are alternatively spliced leading to the inclusion or the exclusion of the transcription transactivation domain. Inclusion of the transactivation domain transforms these tumor-suppressor proteins into oncoproteins [25]. The transcriptional activity of p53 is itself controlled via the alternative splicing of MDM2. Variations in the splicing of MDM2 in cancer tissues influence its accumulation in the nucleus and therefore affect its capacity to repress the transcription of p53 [26, 27]. Other examples of cancer-associated changes in alternative splicing are listed in Table 1.

2.2 Cellular Invasion

Integrins are a family of cell adhesion transmembrane proteins. Their expression modulates the invasive properties of cancer cells. Numerous integrin splice

Table 1 Examples of genes expressing splicing variants that are relevant to various aspects of cell transformation and cancer

Gene	Splice variants	Cancer type	Functional impact	References
Cellular proliferation				
FGFR1 (Fibroblast growth factor receptor 1)	FGFR1-α FGFR1-β FGFR1-IIIb FGFR1-IIIc	Brain, breast, colon and pancreas, PANC-1 cells	FGFR1-β correlates with pancreatic adenocarcinoma cell growth and resistance to chemotherapy. FGFR1-β has a 10-fold higher affinity for the FGF ligand, which may provide a growth advantage to tumors. FGFR1-β is the dominant splice variant found in glial cell and pancreatic tumors. FGFR1-α inhibits adenocarcinoma cell growth in vivo and restores cytotoxic response to chemotherapy. FGFR1-IIIc expressed in pancreatic cancer cells can enhance pancreatic ductal cell transformation.	[202, 220–222]
FGFR2	FGFR2-IIIb FGFR2-IIIc	Prostate, lung metastasis, breast, urothelial, and cervical carcinomas	In prostate cancers, the expression of FGFR2-IIIc is upregulated while that of FGFR2-IIIb is downregulated. Restoration of FGFR2-IIIb to malignant cells expressing FGFR1 represses tumor growth rate and re-establishes responsiveness to stromal cells.	[21–23]
FGFR3	FGFR3-Δ8-10 FGFR3IIIc FGFR3IIIS	Bladder, colorectal cancer, various	FGFR3-Δ8-10 acts as a dominant negative regulator of FGFR3 signaling in bladder cancer. FGFR3IIIS functions as a dominant negative inhibitor of FGFR3-induced growth arrest. FGFR3IIIS may regulate FGF and FGFR trafficking and function, thus contributing to the development of a malignant phenotype.	[223, 224]
FGFR4	FGFR4-s	Gastric, colon, and pancreatic cancer cell lines	The splice variant FGFR4-s encodes a soluble form of the receptor with a ligand binding affinity that is different from the transmembrane FGFR4 receptor.	[225]

(continued)

Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
p53	p53β and p53γ Δ40p53 Δ40p53β and γ Δ133p53 Δ133p53β and γ	Breast cancer, various	N-terminally truncated splice variants (Δ40p53) suppress both transcriptional and growth inhibiting activities of p53. While full-length p53 efficiently induces apoptosis, p53β triggers a more attenuated apoptotic response and Δ133p53β inhibits apoptosis and acts as a dominant negative.	[24]
p63	ΔNp63α	Breast, squamous cell carcinoma of the esophagus	ΔNp63α promotes survival of breast cancer cells by binding to TAp73 and inhibiting its pro-apoptotic activity.	[226]
p73	p73α p73β ΔNp73 TAp73	Prostate, squamous cell carcinoma and adenocarcinoma of the esophagus, various	p73α is abundant in prostate cancer. It represses drug-induced apoptosis in two non-small cell lung cancer cell lines but not in other cancer cell lines. p73α and p73β isoforms have antagonistic functions on drug-induced apoptosis. ΔNp73 impairs the transcriptional activity of TAp73 and p53.	[25, 227, 228]
MDM2	>than 40 different isoforms	Various	Splice variants that lack the p53 binding domain but retain the RING domains are detected in various cancer cell lines and primary tumors. Many negatively regulate the MDM2 full-length protein and activate and upregulate p53. MDM2-b cannot bind p53 but can sequester the full-length MDM2 protein in the cytoplasm. Its overexpression inhibits apoptosis and enhances cell growth in cancer cell lines.	[26, 27, 229, 230]
MDM4 (HDMX)	HDMX-E HDMX-A HDMX-G HMDX211 XALT1 XALT2	Soft-tissue sarcomas	Expression of the HDMX-E splice variant correlates with decreased patient survival and is a stronger negative regulator of p53 than the full-length HDMX protein.	[231, 232]

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Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
PTEN	PTEN-3a, 3b, 3c, PTEN-5a, 5b, 5c, DelE5, DelE6	Cowden syndrome, sporadic breast cancers	SV-5a decreases P-Akt level and cyclin D1 promoter activity, SV-5b and -5c increase Cyclin D1 promoter activity. SV-5b functionally counteracts the activity of PTEN.	[233]
HTERT	Several isoforms	Various	Telomerase is activated in 85–90 % of human tumors, which represents the single most frequent alteration associated with malignancy. Many shorter versions are inactive. hTERT-alpha is one of the four isoforms that are considered negative regulators of telomerase activity.	[234]
Cyclin D1	Cyclin D1a Cyclin D1b	Prostate	Expression of Cyclin D1a inhibits cell-cycle progression in androgen receptor-dependent prostate cancer cells while Cyclin D1b stimulates proliferation. The altered androgen receptor regulatory capacity of Cyclin D1b is associated with an increased risk of prostate cancer.	[235, 236]
CYP1A1 (Cytochrome P450 1A1)	CYP1A1v	Epithelial ovarian	In contrast to the ER localization for the wild-type enzyme, CYP1A1v is restricted to the nucleus and mitochondria. CYP1A1v induces anchorage-independent growth.	[237]
KAP (cyclin-dependent kinase associated protein phosphatase)	Four variants	Glioblastomas	A dominant negative KAP variant increases cell proliferation.	[238]
hMena (ENAH)	hMena(+11a)	Breast and breast cancer cell lines	Overexpression and phosphorylation of hMena (+11a) increases the activation of p42/44 mitogen-activated protein kinase (MAPK) and cell proliferation.	[239]

(continued)

Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
APC	cAPC BS-APC 0.3-APC	Colorectal cancers, SW480 colon cancer cell line	All three splice variants alter cellular morphology and affect cell growth by elongating the G1 phase of the cell cycle. Overexpression of the full-length ACP in SW480 colon cancer cells impairs proliferation while the removal of the truncated APC reduces cell growth. The truncated splice variants enhance the migratory capacity of colon cancer cells. In contrast to 0.3-APC, the c-APC and brain-specific (BS) APC isoforms suppress the tumorigenic phenotype of cells in culture.	[240, 241]
β 1 integrin	β 1C	Endometrial cancers	β 1C acts as a growth modulator in cancer cells.	[28]
Cellular adhesion, invasion and angiogenesis				
CD44	>20 isoforms of CD44v	Gastric carcinomas urothelial cancer, metastatic lesions of gynecological cancers, various	CD44v4-v7 isoforms alter hyaluronate binding. CD44v8-v10 are associated with tumor progression. CD44v6 is involved in the progression of carcinoma, nodal metastasis, myometrial invasion and vascular invasion.	[29, 31, 242]
CD99	CD99wt CD99sh	Osteosarcoma, prostate and breast cancer cells	CD99wt inhibits anchorage-independent growth, migration, and metastasis, whereas the CD99sh markedly elevates motility, binding to fibronectin, MMP-9 expression, and invasiveness of MDA-MB-231 and MCF-7 cells.	[243, 244]
Fibronectin	EDA EDB IIICS-0, -95 and -120	Melanoma, prostate, lung, liver	Preferential inclusion of exon EDB was found in lung cancer and of both the EDB and EDA exons in liver cancer.	[245 246]
C-CAM1 (Cell–cell adhesion molecule)	L-C-CAM1 S-C-CAM1	Colorectal, breast, prostate carcinomas, non-small cell lung cancers (NSCLC) and cell lines	Tumor cells expressing L-C-CAM1 grow more slowly and are less tumorigenic, suggesting that L-form C-CAM1 is a tumor suppressor. NSCLC tissue and cell lines express predominantly S-C-CAM1.	[247, 248]

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Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
CCK$_2$ R (Cholecystokinin-2/ gastrin receptor)	CCK$_2$R ΔCCK$_2$R CCK$_2$LR CCK$_{2i4sv}$R	Esophageal, gastric, pancreatic and colorectal cancers	CCK$_2$R and CCK$_{2i4sv}$R may regulate tumor growth and dissemination. Expression of the CCK$_{2i4sv}$R isoform induces activation of the Src kinase with which it appears to form a signaling complex. CCK$_{2i4sv}$R expression and activation of Src induce tumor cell growth and angiogenesis.	[37]
KLF6	KLF6-SV1 KLF6-SV2 KLF6-SV3	Epithelial ovarian cancer, prostate cancer, glioblastoma	KLF6-SV1 variant promotes cell proliferation and invasion. Its downregulation suppresses tumor formation. KLF6-SV1 and KLF6-SV2 are mislocalized to the cytoplasm and antagonize wtKLF6 function leading to decreased p21 expression and increased cell growth. Both splice variants are upregulated in tumor versus normal prostatic tissue. Decreased KLF6 and increased KLF6-SV1 expression are common primary glioblastomas. These changes have antagonistic effects on the growth of glioblastoma cell lines.	[88, 249, 250]
Rac1	Rac1b	Colorectal and breast cancer cells	The GTPase Rac1b is mostly expressed in its active and GTP-bound state, compromising activation of important signaling pathways that are classically stimulated by Rac1. Rac1b overexpression may facilitate tumor progression by enhancing Dishevelled-3-mediated Wnt signaling pathway and inducing Wnt target genes specifically involved in decreasing the adhesive properties of colorectal cells. Rac1b induces an increase in cellular reactive oxygen species leading to genomic instability and promotion of tumorigenesis.	[251, 252]
OPN (Osteoponin)	OPN-c	Hepatocellular carcinoma	Splice variant OPN-c promotes cellular invasion and correlates with metastatic potential.	[253]

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Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
RON (Macrophage-stimulating protein receptor)	RonD RonD165 RonD160 RonD155	Gastric carcinoma breast and colorectal cancers	RonD mRNA lacks exon 11, which renders the protein constitutively active. RonD induces invasion and mobility properties to cancer cells and participates in malignant transformation of epithelial tumors by activating the epithelial-to-mesenchymal transition.	[33, 125]
HER2 (neu/c-erbB-2) receptor tyrosine kinase	At least five splice variants	Various epithelial tumors	Two variants encode soluble proteins that function as inhibitors of HER2 to inhibit the proliferation of cancer cells. The variant HER2-ECD that contains the extracellular domain is progressively downregulated in more advanced gastric tumors, suggesting that the soluble variants inhibit tumor progression.	[254]
VEGF	VEGF121 VEGF165 VEGF189 VEGF165b	Prostate, kidney, breast, colorectal carcinoma, different solid tumors	In contrast to the other pro-angiogenic variants, VEGF165b is anti-angiogenic. VEGF165b is downregulated in several carcinomas. An increase in the VEGF121/VEGF165-189 ratio enhances angiogenesis in prostate tumors.	[35, 255]
HYAL	HYAL1-v1	Bladder	HYAL expression in bladder cancers promotes tumor growth, invasion, and angiogenesis. HYAL1-v1 expression may negatively regulate bladder tumor growth, infiltration, and angiogenesis.	[256]
PTK2 Focal adhesion kinase (FAK)	FAK-related non-kinase (FRNK)	MTLn3 mammary adenocarcinoma cells	Expression of FRNK inhibits cell spreading and migration, and reduces phosphorylation of FAK.	[257]
Ilp45 (Invasion inhibitory protein 45)	Ilp45S	Infiltrating gliomas	A tumor-specific alternative splicing event generates an aberrant and unstable Ilp45S splice variant that acts as a dominant negative.	[258]

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Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
uPAR (cellular receptor for urokinase-type plasminogen receptor)	uPARdel4/5	Breast	Splice variant uPAR-del4/5 is predominantly expressed in various cancer cell lines and tumors and its expression is associated with shorter survival of cancer patients.	[259]
Resistance to apoptosis				
Fas (CD95)	Fas FasExo6 Del (sFas) FasExo3-4 Del FasExo3-4,6 Del FasExo4 Del FasExo4-6 Del	Lymphoma cell lines hepatocellular carcinoma, bladder, breast, ovarian, renal cell carcinoma, and melanoma	Shorter splice variants of Fas lack the membrane-anchoring transmembrane domain, therefore antagonizing the pro-apoptotic function of full-length FAS. sFas induces resistance to FAS-mediated apoptosis in human lymphoma cell lines. sFas levels increase in the serum of cancer patients and correlates with tumor stage and burden. Increased levels of sFas are observed in several cancers.	[260–263]
APAF1	APAF-1-ALT	LNCaP human prostate cancer cell line	The splice variant shows antagonistic functions during apoptosis and may contribute to resistance to DNA damage-induced treatment.	[264, 265]
BIRC5 (Survivin)	Survivin-ΔEX3, Survivin-2B Survivin-3B	Breast carcinoma, metastatic gastric cancer	Survivin-2B is pro-apoptotic and antagonises Survivin and Survivin-ΔEX3 (both with anti-apoptotic function) that are overexpressed in several tumors. Survivin-ΔEX3 forms a complex with Bcl-2 that inhibits the activity of caspase-3. Survivin-2B is downregulated in breast and gastric cancers.	[266, 267, 268]
Bcl-2	Bcl-2α Bcl-2β	Prostate and colon	Bcl-2α contributes to the resistance of cancer cells to chemotherapeutic drugs. Bcl-2β has minimal or no anti-apoptotic function.	[269, 270]

(continued)

Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
Bcl-x	Bcl-xL Bcl-xS Bcl-xΔTM Bcl-xβ Bcl-xγ	Prostate, ovarian, leukemias	Bcl-xL is the major anti-apoptotic splice variant expressed in various tumors rendering cancer cells more resistant to chemotherapeutic drugs. Bcl-xS acts as the pro-apoptotic isoform, antagonizing the anti-apoptotic properties of Bcl-xL and sensitizing cancer cells to chemotherapeutic drugs. Bcl-xΔTM, Bcl-xβ, and Bcl-xγ exhibit various levels of anti-apoptotic activity.	[49, 271, 272]
Bax	Bax-α Bax-β Bax-γ Bax-δ Bax-ε Bax-σ Bax-ω	Breast Hodgkin lymphoma	Bax-α, the most frequent splice variant, and Bax-σ act as pro-apoptotic proteins, while Bax-ω has anti-apoptotic activity. Higher levels of Bax-α and lower levels of Bcl-2 in breast cancer tissue correlate with lower tumor grade. Bax-β may promote cancer cell growth. Bax-γ is considered a negative regulator of other Bax isoforms. Bax-ε induces apoptosis when expressed ectopically.	[273–275]
c-FLIP	c-FLIPL c-FLIPS c-FLIPR	Colorectal cancer	c-FLIPL acts as a pro-apoptotic isoform while c-FLIPR is anti-apoptotic. c-FLIPL but not c-FLIPS can inhibit cell death induced by anticancer drugs.	[276, 277]
IG20 (Insulinoma-Glucagonoma)	IG20pa MADD IG20-SV2 DENN-SV	HeLa and PA-1 cancer cell lines	IG20-SVs play critical role in cell proliferation and apoptosis. IG20pa renders cells more susceptible to apoptosis and decreases cell proliferation while DENN-SV can increase the resistance to apoptosis and cell proliferation. MADD demonstrates antagonistic functions during apoptosis. MADD is sufficient and necessary for cancer cell survival.	[67, 278]
Mcl-1 (Myeloid cell leukemia)	MCL1-L MCL1-S	Leukemia	MCL1-L is the anti-apoptotic while MCL1-S is pro-apoptotic.	[279]

(continued)

Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
Bim	BimL BimEL BimS Bimγ	Prostate, precursor B cell acute lymphoblastic leukemia cell line	All Bim isoforms promote apoptosis. BimS is the most potent pro-apoptotic isoform. BimL induces apoptosis by binding to and antagonizing anti-apoptotic Bcl-2 family members, such as Bcl-xL and BCL2L2. Bimγ inhibits clonal growth in prostate cancer cells. Induction of Bim by glucocorticoids is required for the complete apoptotic response in a precursor B cell acute lymphoblastic leukemia cell line.	[280–282]
CASP2	CASP-2L CASP-2S CASP-2L-Pro CASP-2S-Pro	Human B lymphoma, Namalwa and U937 human leukemic cells	CASP-2L is the most abundant pro-apoptotic splice variant while CASP-2S can function as anti-apoptotic isoform. CASP2-L expression sensitizes non-small cell lung cancer to cisplatin-induced apoptosis. CASP-2L-Pro functions as an endogenous apoptosis inhibitory protein that antagonizes caspase activation and cell death.	[283, 284]
CASP3	CASP-3S	Breast carcinomas and cancer cell lines	CASP-3S inhibits apoptosis and is associated with chemoresistance.	[285]
CASP8	CASP-8a CASP-8b CASP-8L	Neuroblastoma cell lines	CASP-8L and CASP-8a are dominant negative splice variants of CASP8. CASP-8b is the pro-apoptotic variant.	[286, 287]
CASP9	CASP-9a CASP-9b	LN-229 astrocytoma cells	CASP9a is the initiator caspase of the mitochondrial apoptotic pathway. CASP9b is the anti-apoptotic splice variant. Transient overexpression of CASP9b protects LN-229 astrocytoma cells from CD95 ligand-mediated apoptosis.	[288]

(continued)

Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
FIR (FUSE-binding protein-interacting repressor)	sv-FIR	Primary colorectal cancers	Splice variant sv-FIR promotes tumor development by disabling FIR repression and inducing high levels of c-MYC to counteract apoptosis in colorectal cancer.	[57]
WT1 (Wilms tumor 1)	WT1(+KTS) WT1(−KTS)	Osteosarcoma nephroblastoma Wilms tumor, leukemia	In osteosarcoma cell lines expressing low levels of the wild-type transcript, inducible expression of WT1, particularly the (−KTS) isoform, triggers an initial G1 cell cycle arrest, followed by apoptosis. The WT1(−KTS) isoform appears to mediate transcriptional activation of genes implicated in cellular differentiation, possibly also repressing proliferation-associated genes. WT1(+KST) appears to regulate cell cycle and inhibits apoptosis.	[289, 290]
CK8 (Cytokeratin 8)	AS-CK8	Lung, non-small cell lung cancer cell lines and primary tumors	AS-CK8 lacks the caspase cleavage site. Cancer cells expressing this splice variant may have increased resistance to apoptosis.	[291]
Multidrug resistance				
MRP1	>> 20 splice variants	Ovarian tumor	Some of the splice variants confer resistance to doxorubicin.	[59]
M MAD2	MMAD2β	Human gastric adenocarcinoma cell line SGC7901	MAD2β can increase multidrug resistance in SGC7901 cells.	[292]
DNA excision/repair				
BRCA1	> than 12 splice variants	Breast and ovary	The nuclear localization signal is absent in some of the splice variants. Disruption of BRCA-1 pre-mRNA splicing patterns contributes to cell growth and development of breast and ovarian cancers.	[86, 293, 294]

(continued)

Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
BRCA2	Δ12-BRCA2	Breast	Overexpression of the Δ12-BRCA2 splice variant is associated with steroid receptor-negative tumors.	[295]
XPC	XPC variant lacking exon 12	Xeroderma pigmentosum	This splice variant has diminished DNA repair function and may contribute to cancer susceptibility.	[296]
Miscellaneous				
Naf1	Naf1FL Naf1 α2 Naf1 β2 Naf1 α3 Naf1 β3 Naf1 α4	Acute myeloid leukemia (AML) leukemia-lymphoma cell lines	Naf1 α2 is the main transcript. Naf1FL and Naf1 α2 have equal NF-κB inhibitory effects, while Naf1 α4 is less effective. Naf1 α3 has a dominant negative effect in AML patients.	[297]
IRF-1 (Interferon regulatory factor-1)	Variants lacking some combinations of exons 7, 8, and 9	Cervical cancer	Splice variants with deletions of functional domains leading to differential transcriptional activities.	[298]
SRA1 (Steroid receptor RNA Activator 1)	SRAP	Breast cancer cell lines	Decreased estrogen receptor activity in breast cancer cells suggesting an antagonistic role for SRAP.	[299]
Syk (Spleen tyrosine kinase)	Syk(S)	Breast	Expression of Syk(S) occurs frequently in primary breast tumors and may contribute to mammary tumor progression.	[300]
WNT2B	WNT2B2	Primary gastric cancer	WNT2B2 upregulation may lead to carcinogenesis through activation of the β-catenin-TCF signaling pathway.	[301]

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Table 1 (continued)

Gene	Splice variants	Cancer type	Functional impact	References
Met (Met tyrosine kinase receptor)	Met-SM	NIH3T3 mouse fibroblasts	Met-SM may contribute to development and progression of human cancer.	[302]
ER (Estrogen Receptor)	ERβ1 ERβΔ125 ERβΔ1256	MDA-MD-231 breast cancer cells	ERβΔ1256 increases cell sensitivity toward lower concentrations of tamoxifen, ERβ1 and ERβΔ125 weaken the growth-inhibitory effect of tamoxifen.	[303]

*Although many examples are provided, the list is not exhaustive and only selected references are included

variants that either facilitate or inhibit cellular proliferation have been described [28]. The cell–cell adhesion glycoprotein CD44 whose expression is associated with the metastatic potential of cancer cells [29] is produced in more than 20 splicing isoforms. Isoforms that contain exon v6 induce metastasis in mammary and pancreatic carcinomas in rats [30], while inclusion of exon v10 modifies CD44 adhesion properties and contributes to cancer progression [31]. Integrins often interact with fibronectin, itself produced in various versions. The alternate EDB- and EDA-containing isoforms of fibronectin are involved in cell adhesion and spreading [32]. In breast and colon cancers, overexpression of RonD, the splice variant of the transmembrane receptor MSF (macrophage-stimulating factor) is overexpressed, which enhances the migratory properties of cancer cells [33]. Table 1 lists additional genes whose change in alternative splicing has been shown to affect cell adhesion properties and invasiveness.

2.3   Angiogenesis

Several splicing changes affect angiogenesis, most notably those of the vascular endothelial growth factor (VEGF) in which an alternative 3′ splice site causes an antiangiogenic form to be produced in normal tissues [34]. Recently, it was also shown that prostate tumors have reduced incorporation of exon 7. Experimental inhibition of exon 7 inclusion reduced the ability of transplanted cells to induce angiogenesis in mice [35]. Exon 7 incorporation is positively regulated by the RNA-binding protein T-STAR (also known as SLM-2) [36]. VEGF expression is itself affected by the alternative splicing products of the Estrogen receptor alpha and the cholecystokin-2/gastrin receptor ($CCK_2 R$) [37, 38]. Another relevant event is the removal of exon 3 of Survivin, which gives it a specific function in promoting angiogenesis [39].

2.4   Resistance to Apoptosis

Alternative splicing has a strong impact on the function of proteins implicated in apoptosis. A comprehensive inventory of isoforms derived by alternative splicing of apoptotic genes and a summary of their known functions appeared recently [40]. The functional consequences of alternative splicing have been demonstrated for the death receptor Fas, as well as the adaptor and regulatory proteins APAF1 and Survivin. The function of apoptotic mediators such as Bcl-x, Bfk, Mcl1, Bim and Bid, and several caspases were also shown to be modulated by alternative splicing [40]. The alternative splicing of these genes produces isoforms that often have different and sometimes opposing activities.

A close association with cancer is particularly well documented for the splicing variants of Bcl-x. Competing 5′ splice sites dictate the expression of the $Bcl-x_L$ and $Bcl-x_S$ isoforms, two proteins with antagonist regulatory functions [41]. $Bcl-x_L$ is an anti-apoptotic protein that protects the integrity of mitochondria, and is overexpressed

in many types of cancer cells [42–46]. On the other hand, the pro-apoptotic isoform Bcl-x$_S$ can heterodimerize with Bcl-x$_L$ to abrogate its anti-apoptotic activity [47]. Furthermore, Bcl-x$_L$ overexpression is directly responsible for the resistance of cancer cells to stresses and chemotherapeutic drugs [41, 48–56].

Another example of a splicing event linked to apoptosis is FIR, a known inhibitor of MYC. In this case, a shorter splice isoform of FIR switches protein activity to facilitate the overexpression of MYC, inhibiting apoptosis [57]. The alternative splicing of protein kinase C delta (PKCδ) was also shown to produce an isoform that can protect human teratocarcinoma cells from apoptosis [58]. Other recent examples of splice isoforms affecting apoptosis are presented in Table 1.

2.5   Multidrug Resistance

Drug transporters defend cells against cytotoxic agents. Thus, alterations in their alternative splicing may alter the efficacy of anticancer agents. One major group of transporters are known as the multidrug resistance-associated proteins (MRP; ABCC gene family). Alternative splicing of many genes in this group creates functional variants, as is the case with MRP1 that produces splicing variants conferring resistance to doxorubicin in ovarian tumors [59]. Alternative splicing of MRP4, a drug-efflux pump mediating the efflux of nucleotide analogs, generates a non-functional protein via introduction of premature termination codons [60]. Differential expression of two mRNA isoforms of the ATP-binding cassette transporter gene ABCB 5 was reported in melanoma cells [61]. Several alternatively spliced P-glycoprotein transcripts have been found in multidrug-resistant cells and their expression correlates with drug resistance [62, 63]. Finally, BCRP (ABCG2) splice variants are differentially expressed in human drug-selected breast cancer cell lines [64].

The deregulated expression of dominant negative variants of p63 and p73 lacking the transactivation domain can inhibit the transactivation of target genes and apoptosis, thus contributing to chemoresistance [65]. A splice variant of the spindle checkpoint gene Mad2 abrogates mitotic arrest and adriamycin-induced apoptosis [66]. Finally, a MAP-kinase activating death domain (MADD) splice variant of the IG20 gene suppresses tumor cell survival and enhances susceptibility to apoptosis and anticancer drugs [67].

3   Alternative Splicing Control: Basic Principles

Before discussing specific examples of molecular alterations responsible for cancer-specific splicing patterns, it is important to review some key principles of splice site control and selection. Splice site selection is determined first by the sequence of splice sites. The sequence of constitutive splice sites that are used

all the time usually matches the consensus CAG/GUGAGU, for a 5′ splice site («/» indicates the junction between exon and intron), and a polypyrimidine-rich tract followed by CAG/at the 3′ splice site (Fig. 2a). In contrast, alternative splice sites usually display various mismatches to the consensus sequence, which make them intrinsically weaker splice sites. This intrinsic weakness renders them amenable to modulation by control factors that may increase or decrease their use. Hence, the use of alternative splice sites is often influenced by the presence of nearby exonic or intronic auxiliary elements that promote or repress splicing. These sequence elements function by recruiting proteins that either directly modulate splice site recognition, affect the formation of specific splicing complexes or change the conformation of the pre-mRNA to promote the use of certain splice site combinations.

Direct modulation of splice site recognition is promoted by members of the SR family of proteins, which are frequently associated with stimulatory elements. However, other types of proteins with stimulatory activity have been identified (for example, CELF, hnRNP H, hnRNP L, TIA1, and TIAR) [68]. When these proteins are bound in the proximity of a splice site, they may interact with other components of the splicing machinery (U1 snRNP at the 5′ splice site or U2AF at the 3′ splice site) to improve their binding (Fig. 2b). Alternatively, some sequence elements located near splice sites may repress their use (Fig. 2c) by recruiting proteins that hinder the recognition or utilization of the adjacent splice sites.

In addition to the direct role of proteins recruited in the vicinity of a splice site, changes in the conformation of the pre-mRNA may also influence splicing decisions. These changes sometimes involve the formation of a secondary structure that blocks the utilization of a splice site [69]. In other cases, the changes are induced by an interaction between proteins bound at different locations on a pre-mRNA. Such spatial rearrangement can stimulate the use of a splice site located outside of the loop, and may simultaneously repress splice sites located inside that loop (Fig. 2d). Although this model was initially proposed to explain the mechanism of action of the hnRNP A1 protein in alternative splicing [70–72], it is also relevant to the mechanism of action of hnRNP H, and possibly hnRNP I/PTB and Nova-1 proteins [72–75]. hnRNP A1 may also counteract the activity of certain SR proteins through a different model which proposes that hnRNP A1 can nucleate and spread over regions of a pre-mRNA through cooperative RNA binding [76, 77].

Detailed investigations of selected alternative splicing events indicate that splice site selection is determined by a combination of several layers of positive and negative regulators. Thus, the frequency with which a given alternative splicing pattern is used will be determined by: (1) the intrinsic strength of each splice site involved, (2) the number, identity, and the position of control elements, and (3) the relative concentration and affinity of each RNA-binding protein to its respective binding site. The phosphorylation of specific SR and hnRNP proteins will also have an impact on splice site selection since this modification can affect their activity and cellular distribution [78].
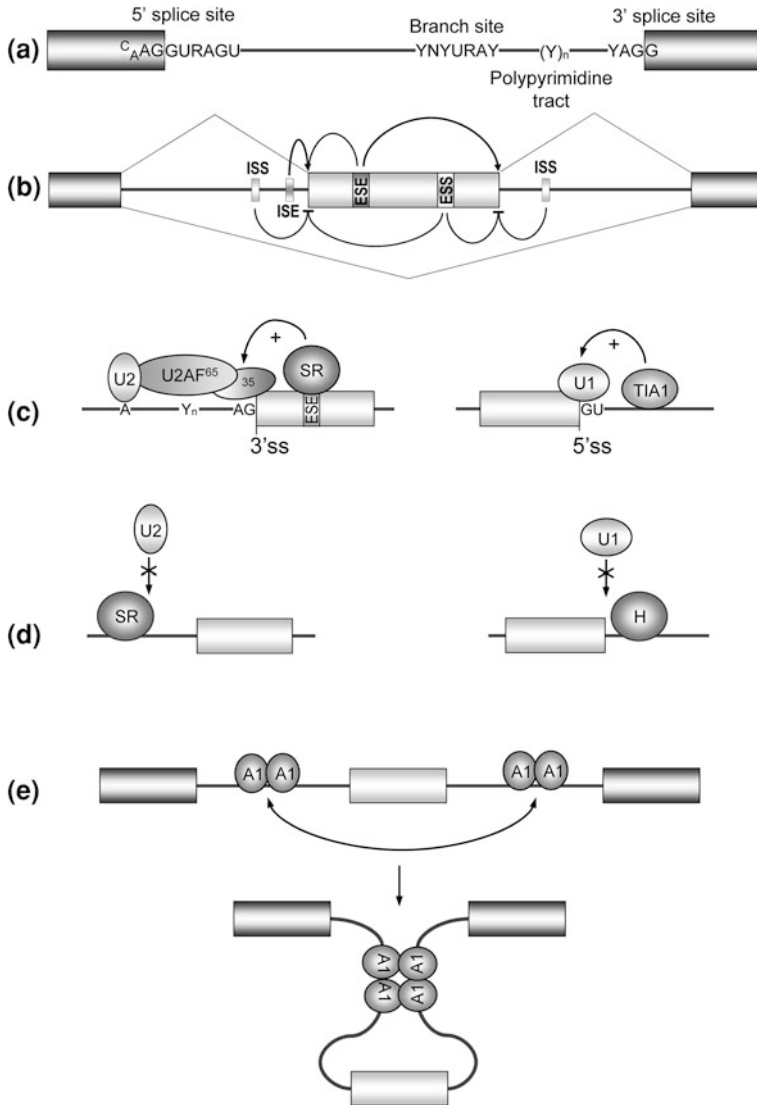
4 Molecular Basis for Splicing Alterations in Cancer

Different types of molecular perturbations can cause alterations in alternative splicing profiles. Point mutations at splice sites have been linked to numerous diseases and have been proposed to account for 15 % of human genetic diseases [2, 79]. This number is likely to be considerably larger since mutations in introns, which constitute the majority of the gene sequence, are rarely considered. A recent

◄**Fig. 2** Control of splice site selection. **a** Schematic representation of a splicing unit (*top*) and an alternative splicing unit (*bottom*). The consensus sequences of a 5′ splice site, a branch site and a 3′ splice site are shown. *R* purine, *Y* pyrimidine. Elements that control splicing decisions are shown and include positively acting exonic and intronic splicing enhancers (ESEs and ISEs, respectively), as well as negatively acting exonic and intronic splicing silencers (ESSs, ISSs, respectively). **b** Proteins bound to exonic or intronic enhancers can recruit or stabilize the binding of splicing factors to the 3′ (*left*) or 5′ (*right*) splice site. In the illustrated examples, an SR protein bound to an ESE enhances U2AF and U2 snRNP binding to the 3′ splice site/branch site region [102, 217], while binding of TIA1 to ISE can stimulate recognition of an adjacent upstream 5′ splice site by the U1 snRNP [126]. **c** Splicing inhibition. Steric interference caused by the presence of a protein bound in the proximity of a splice site impedes its recognition. The SR and hnRNP H proteins can, respectively, inhibit the binding of the U2 and U1 snRNPs [218], [219]. **d** Change in pre-mRNA conformation can be promoted by an interaction between hnRNP A1 proteins bound to introns flanking an alternative exon, provoking repression of the looped out exon, and stimulation of splicing between the distal pair of exons [72]

study suggests that as much as 60 % of the point mutations that cause genetic diseases affect splicing decisions [80]. Thus, we can anticipate that several cancer-related genes sustain mutations at splice sites or in regions bound by proteins that control the selection of splice sites. Exonic mutations that create a stop codon can also affect the function of a splicing control element, thereby modifying splicing profiles [81]. In other cases, missense mutations, again through changes in splicing, may have a more profound impact on protein structure than the predicted change in amino acids caused by the mutations. Finally, even mutations that are considered to be neutral because they do not change amino acids can have an impact on alternative splicing [2, 3]. The following sections present some examples of mutations (Fig. 3) and changes in the expression of splicing factors (Fig. 4) that affect the splicing of cancer-associated genes.

4.1   Mutations at Splice Sites and in Auxiliary Elements

According to the Cancer Genome Project of the Welcome Trust Sanger Institute (http://www.sanger.ac.uk/genetics/CGP/Census/), about 363 human genes have mutations that have been associated with cancer; 90 % of these genes have somatic mutations, approximately 20 % show germline mutations that predispose to cancer, and 10 % show both somatic and germline mutations. Specifically, 42 of these 363 genes have sustained mutations that affect splicing. Splicing mutations are the most prevalent type of mutations found in the NF1 gene, which is implicated in neurofibromatosis, the most common form of autosomal dominant cancer in humans [82]. Mutations in the splice sites of NF2 are used as markers to grade the severity of the disease [83].

Similarly, approximately 30 mutations affecting the splice sites of p53 have been reported in different cancers [84]. Another interesting example concerns the tumor suppressor gene APC, where two mutations have been closely associated with the development of familial adenomatous polyposis [85]. One of these mutations creates a splice site that promotes the deletion of one nucleotide resulting in the production of a truncated APC protein. Other mutations that

weaken or create new splice sites in different genes associated with cancers are listed in Fig. 3. This list includes well-known genes such BRCA1, BRCA2, CDKN2, PTEN, KIT, ATM, and XPC.

Mutations in *cis*-acting splicing regulatory elements can also modify the relative abundance of splicing variants or induce the utilization of new splice sites. For example, a mutation of an exonic element in the BRCA1 gene reduces the binding of ASF/SF2, thereby increasing the exclusion of exon 18 [86]. Many mutations that affect splicing control elements have been described in the BRCA1 gene [87]. A polymorphism in the intron upstream of exon 2 in KLF6 creates a binding site for SRp40 that activates cryptic sites in exon 2 [88]. This mutation is associated with an increased risk of prostate cancer. The altered KLF6 proteins may counteract the tumor-suppressor activity of the wild-type protein. Intronic and exonic mutations in the cadherin CDH17 gene that do not directly affect the splice junctions have been proposed to interfere with splicing decisions [89]. Other examples of this type of mutation have been reported in NF1, APC, and MLH1 genes (Fig. 3).

The diversity of splicing control elements suggests that most intronic or exonic mutations that were initially considered silent mutations may in fact have an important impact on constitutive and alternative splicing. For the same reasons, a large subset of single nucleotide polymorphisms (SNPs) found within the human population may modulate alternative splicing. For example, a SNP in an SRp40 binding site in the APC gene correlates with exon omission and attenuated familial adenomatous polyposis [90]. These differences may predispose some individuals to develop certain types of cancers, while other polymorphisms may increase their resistance. Consistent with this view, a recent study has identified a high frequency of alternative splicing in microsatellite regions linked with human longevity or resistance to anticancer treatments [91].

4.2   Alterations in the Activity of Splicing Proteins

While many *cis*-acting mutations have been shown to affect splicing, the majority of alterations in alternative splicing profiles appear to proceed from changes in the expression or activity of splicing factors [4]. Because a proof for the direct contribution of such changes to cancer is not always available, only experimentally proven cases will be discussed below.

4.2.1   Chromosomal Translocations

In some cancers, as in Ewing's sarcomas, chromosomal translocations affect the genes EWS and TLS (FUS or hnRNP P2) that encode RNA-binding proteins. The interaction of these proteins with splicing factors such as SF1, U1C, YB-1 and some SR proteins may explain why their translocation has an impact on splicing control [92–95]. Translocations between these genes and transcription factors of the ETS family result in the production of chimeric proteins with strong transcriptional activity and oncogenic properties, as for example EWS-FLI1,
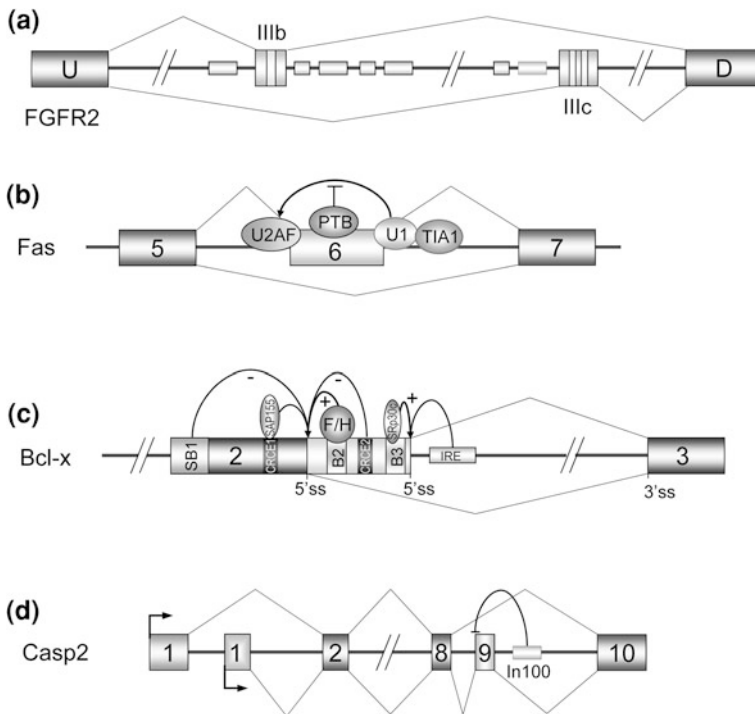
Fig. 3 Alternative splicing of selected cancer-related genes (see main text for details)

EWS-NOR1, and TLS-ERG [96]. The failure of the hybrid proteins EWS-FLI1 and TLS-ERG to recruit the YB-1 and SR proteins may compromise their role in splicing [95, 97]. The interaction of EWS-FLI1 and TLS-ERG with transcriptionally active form of RNA polymerase II [94, 95, 98] may affect the cotranscriptional selection of splice sites. EWS-NOR1 can interact with the snRNP protein U1C. EWS-NOR1 can also enhance the use of a distal 5′ splice site in a reporter pre-mRNA more efficiently than the EWS protein alone [99]. In leukemia, the well-known Bcr-Abl translocation has been associated with splicing defects in SLP65, Bruton's tyrosine kinase, Pyk2 and Ikaros. However, the mechanism responsible for these alterations may be indirect since Bcr-Abl enhances the expression of SRPK1, a kinase that modulates the activity of SR proteins [100].

4.2.2 Alterations in the Expression and Localization of Splicing Control Factors

Viral transformation was first associated with the upregulation and increased activity of SR proteins [101], and this change affected fibronectin pre-mRNA splicing [102]. The first case of a deregulation in the expression of hnRNP and SR proteins in cancer cells was observed in colon adenocarcinomas [103]. Since then, alterations in the expression of a variety of RNA-binding proteins have been
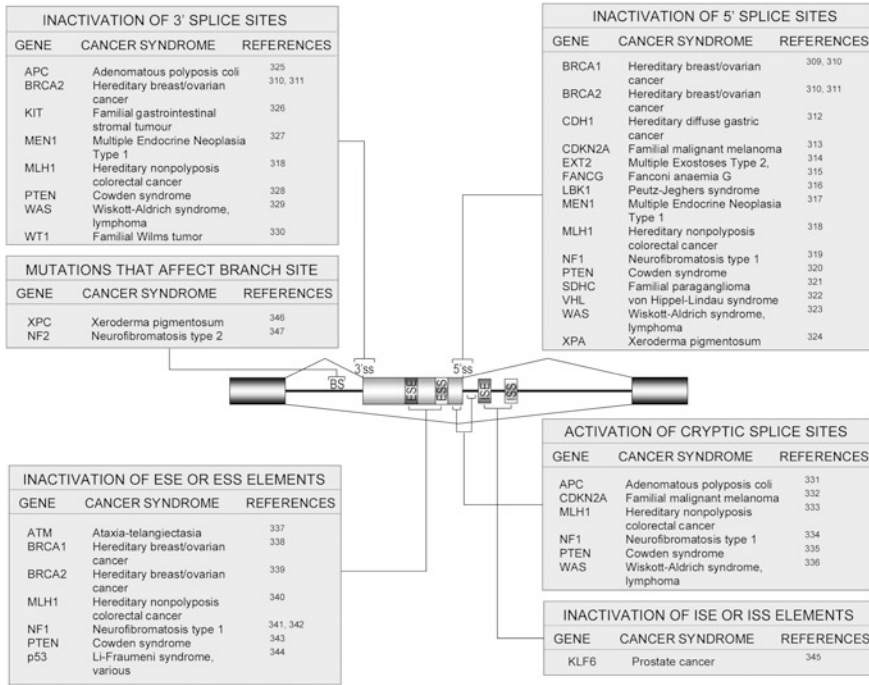
INACTIVATION OF 3' SPLICE SITES

GENE	CANCER SYNDROME	REFERENCES
APC	Adenomatous polyposis coli	325
BRCA2	Hereditary breast/ovarian cancer	310, 311
KIT	Familial gastrointestinal stromal tumour	326
MEN1	Multiple Endocrine Neoplasia Type 1	327
MLH1	Hereditary nonpolyposis colorectal cancer	318
PTEN	Cowden syndrome	328
WAS	Wiskott-Aldrich syndrome, lymphoma	329
WT1	Familial Wilms tumor	330

MUTATIONS THAT AFFECT BRANCH SITE

GENE	CANCER SYNDROME	REFERENCES
XPC	Xeroderma pigmentosum	346
NF2	Neurofibromatosis type 2	347

INACTIVATION OF 5' SPLICE SITES

GENE	CANCER SYNDROME	REFERENCES
BRCA1	Hereditary breast/ovarian cancer	309, 310
BRCA2	Hereditary breast/ovarian cancer	310, 311
CDH1	Hereditary diffuse gastric cancer	312
CDKN2A	Familial malignant melanoma	313
EXT2	Multiple Exostoses Type 2,	314
FANCG	Fanconi anaemia G	315
LBK1	Peutz-Jeghers syndrome	316
MEN1	Multiple Endocrine Neoplasia Type 1	317
MLH1	Hereditary nonpolyposis colorectal cancer	318
NF1	Neurofibromatosis type 1	319
PTEN	Cowden syndrome	320
SDHC	Familial paraganglioma	321
VHL	von Hippel-Lindau syndrome	322
WAS	Wiskott-Aldrich syndrome, lymphoma	323
XPA	Xeroderma pigmentosum	324

INACTIVATION OF ESE OR ESS ELEMENTS

GENE	CANCER SYNDROME	REFERENCES
ATM	Ataxia-telangiectasia	337
BRCA1	Hereditary breast/ovarian cancer	338
BRCA2	Hereditary breast/ovarian cancer	339
MLH1	Hereditary nonpolyposis colorectal cancer	340
NF1	Neurofibromatosis type 1	341, 342
PTEN	Cowden syndrome	343
p53	Li-Fraumeni syndrome, various	344

ACTIVATION OF CRYPTIC SPLICE SITES

GENE	CANCER SYNDROME	REFERENCES
APC	Adenomatous polyposis coli	331
CDKN2A	Familial malignant melanoma	332
MLH1	Hereditary nonpolyposis colorectal cancer	333
NF1	Neurofibromatosis type 1	334
PTEN	Cowden syndrome	335
WAS	Wiskott-Aldrich syndrome, lymphoma	336

INACTIVATION OF ISE OR ISS ELEMENTS

GENE	CANCER SYNDROME	REFERENCES
KLF6	Prostate cancer	345

Fig. 4 Specific examples of mutations reported to affect alternative splicing in cancer. Mutations are organized according to their impact on the inactivation or activation of splice sites by directly changing the structure of the splicing signals or control elements. The name of the gene that is affected, the type of cancer in which it was reported, and relevant references are given

reported in different types of cancers [104] (see Table 2). For example, expression of the SR protein ASF/SF2 is upregulated in many types of cancers and can elicit the transformation of immortal rodent fibroblasts [105]. Notably, ASF/SF2 shifts the alternative splicing of three pre-mRNAs: BIN1 produces an isoform that lacks tumor–suppressor activity; a MNK2 kinase variant can phosphorylate eIF4E in a MAP kinase-independent manner; the S6K1 kinase is spliced to produce an oncogenic isoform [105]. Since ASF/SF2 overexpression in breast cancer has been linked to cell motility through the production of RonD [33], ASF/SF2 may contribute to early and late steps of carcinogenesis.

While over expression of SR proteins has been linked to cancer, downregulation and altered phosphorylation are relevant to apoptosis, a process that is often defective in cancer cells. A reduction in ASF/SF2 can induce apoptosis. Genomic DNA fragmentation is blocked because the drop in ASF/SF2 changes the alternative splicing profile of the ICAD nuclease to favor the expression of the inactive isoform [106]. Similarly, induction of the apoptotic pathway through activation of the death receptor Fas dephosphorylates SR proteins [107]. Fas and the anticancer drug gemcitabine increase the levels of ceramide that activates protein phosphatase 1 (PP1), which in turn can act on SR proteins. Since ceramide

Table 2 Altered expression of splicing factors associated with a functional impact in cell growth

Splicing factor	Cancer type	Functional impact	References
SR proteins	Colon adenocarcinomas	mRNA levels of different SR proteins are usually lower in tumor samples compared to nonpathological tissues surrounding the tumor. This reduction is more drastic in patients showing an important alteration in CD44 splicing and may be related to the development of metastases	[103]
Tra2β1	Breast	A significant induction of Tra2β1 is found in invasive breast cancer. Given the role Tra2β1 in the alternative splicing of CD44, Tra2β1 expression may affect tumor progression and metastasis	[123]
SRp55	Breast	Higher expression of SRp55 protein is associated with an altered pattern of CD44 variants incorporating exon v7	[304]
SC35 and ASF/SF2	Ovarian	SC35 and ASF/SF2 are induced in ovarian cancer tissues. Their altered expression may be linked with changes in the alternative splicing of CD44	[117]
SF2/ASF	Breast, colon	SF2/ASF promotes the production of the RonD isoform to regulate cell motility and locomotion. Increased SF2/ASF expression is also associated with the redistribution of β-catenin, reorganization of cytoskeleton, and downregulation of E-cadherin. SF2/ASF may play a role in regulating malignant transformation by inducing a RonD-mediated epithelial to mesenchymal transition. SF2/ASF is upregulated in various tumors. SF2/ASF controls the alternative splicing of tumor suppressor BIN1 and the kinases MNK2 and S6K1 favoring splicing of BIN1 isoforms that lack tumor-suppressor activity. Thus the splicing factor SF2/ASF can act as an oncoprotein. Knockdown of ASF/SF2 promotes apoptosis	[33] [105] [106]
SRm160	Various	Upon Ras activation, overexpression of SRm160 stimulates expression of the CD44 isoforms that contain exon v5. Such isoforms correlate with enhanced malignancy and invasiveness	[122]

(continued)

Table 2 (continued)

Splicing factor	Cancer type	Functional impact	References
SRPK1 (Serine-arginine protein kinase 1)	Breast, colon, dysplastic and neoplastic pancreatic ductal cells	SRPK1 is overexpressed in breast and colon cancers and its expression increases with tumor grade levels. Knockdown of SRPK1 in breast and colon cancer cell lines increases the gemcitabine and cisplatin-induced apoptotic response	[174]
PTB (hnRNP I)	Glioblastomas	The expression of PTB in malignant glioblastomas is increased relative to glial cells. This overexpression correlates with increased skipping of the FGFR1α exon	[115]
PTB and SRp20	Ovarian	Both PTB and SRp20 are overexpressed in most ovarian tumors. This overexpression correlates with the increased number of splice variants of MRP1	[59]
hnRNP A1/A2	Various	The expression of hnRNP A1/A2 proteins is elevated in a variety of cancers, whereas A1/A2 expression is lower in normal tissues. Knockdown of hnRNP A1/A2 proteins induces apoptosis of cancer cell lines but not mortal cell lines. hnRNP A1 has been implicated in the alternative splicing of CD44 and Bcl-x	[120], [305–308]
SRPK1 (Serine-arginine protein kinase 1)	Breast, colon, dysplastic and neoplastic pancreatic ductal cells	SRPK1 is overexpressed in breast and colon cancers and its expression increases with tumor grade levels. Knockdown of SRPK1 in breast and colon cancer cell lines increases the gemcitabine and cisplatin-induced apoptotic response	[174]

*Only selected examples are listed

promotes a shift toward the production of the pro-apoptotic Bcl-x$_S$ protein, a reduction in phosphorylation of SR proteins may elicit apoptosis.

An increase in the levels of hnRNP I/PTB and SRp20 in ovarian cancers that are resistant to doxorubicin correlates with the overexpression of some isoforms of the multidrug resistance protein MRP1 [59]. Moreover, PTB knockdown suppresses growth and invasiveness [108]. Overexpression of the alternative splicing factor SPF45 is often observed in tumors and may confer resistance to chemotherapeutic agents [109].

Components of the constitutive splicing machinery may also be subject to differential regulation in tumors. The branchpoint binding protein SF1 is down-regulated in mouse intestinal tumorigenesis. Expression of SF1 is regulated by β-catenin and it affects alternative splicing of several genes including WISP1, FGFR3 and the Estrogen receptor-beta [110]. In pancreatic tumors, the expression of U2AF35 (part of the other main protein complex that recognizes 3′ splice sites) is also often repressed. This situation promotes the synthesis of a constitutively active isoform of the CCK-B receptor and a resulting stimulation of cellular proliferation [111]. The breast cancer-associated scaffold attachment factor SAFB1 interacts with a plethora of splicing factors and affects splicing [112].

In general, however, while several studies have reported increased expression of specific RNA-binding proteins in cancer tissues, the functional impact of these differences in the alternative splicing of important genes in cancer remains poorly documented. A few of the most important cases where this link has been established are presented below.

FGFRs. Approximately 10 distinct elements control the alternative splicing of the mutually exclusive IIIb and IIIc exons in FGFR2 (Fig. 4a). Fox-2 and hnRNP I/PTB proteins have been implicated in the activity of some of these controlling elements [113, 114]. hnRNP I/PTB also controls the alternative splicing of FGFR1. Interestingly, the high levels of hnRNP I/PTB in glioblastomas may promote the exclusion of exon α, thereby improving its affinity for the ligand [115].

*CD*44. An increase in the expression of some SR proteins is associated with the progression from the pre-neoplastic to the metastatic status of mammary tumors [103, 116]. This observation correlates with modifications in the alternative splicing of the cell-surface glycoprotein CD44 in human mammary tumors [103]. In ovarian cancers, an increase in the expression of SR proteins and their hyper-phosphorylated forms has also been observed [117]. In addition, exon v9 of CD44 contains a stimulatory element that can be bound by low molecular weight SR proteins [118].

Sam68 and hnRNP A1 also contribute to the alternative splicing of CD44 by controlling the inclusion of exon v5 [119, 120]. A protein of the same family as Sam68, T-STAR (SLM2), can also bind to the v5 exon to promote its inclusion and this is antagonized by other RNA-binding proteins such as SRp30c, hnRNP G, and SAF-B, as well as by SIAH-1, a ubiquitin ligase and candidate tumor suppressor [121]. The inclusion of exon v5 is stimulated by the phosphorylation of Sam68 following activation of the Ras signaling pathway. This stimulation requires the participation of the splicing co-activator SRm160, which interacts with Sam68

[122]. As demonstrated in a recent study, the binding of Tra2β to exon v4 of CD44 and its synergic action with the nucleic acid-binding protein YB-1 enhance the inclusion of both exons v4 and v5 [123].

Interestingly, overexpression of Brm, a component of the SWI/SNF chromatin remodeling complex can promote the inclusion of the v5–v9 exons through a process that requires Sam68 [124].

Ron. The binding of the SR protein ASF/SF2 to a sequence of exon 12 on the pre-mRNA of Ron, a tyrosine kinase transmembrane receptor, improves the utilization of its 3′ splice site and enhances the exclusion of the immediate upstream exon 11. Upregulation of ASF/SF2 in cancer cells is associated with enhanced exon 11 skipping and generation of RonD, an isoform that improves the motility and invasive properties of these cells. RonD is upregulated in metastatic breast and colon cancers [33, 125].

Fas. Alternative splicing of the pre-mRNA encoding the cell death receptor Fas generates two isoforms through inclusion or skipping of exon 6. The membrane-bound long isoform activates the extrinsic apoptotic pathway, while the soluble short isoform that lacks the transmembrane domain is an anti-apoptotic factor. The production of the long isoform is facilitated by TIA1 and TIAR that bind to a U-rich sequence situated immediately downstream of the 5′ splice site of alternative exon 6. These proteins stimulate the binding of the U1 snRNP at this 5′ splice site (Fig. 4b) [126]. In contrast, the exclusion of exon 6 is enhanced by the binding of hnRNP I/PTB to exon 6 which interferes with the communication between the downstream U1 snRNP bound to the 5′ splice site and U2AF bound to the upstream 3′ splice site [127].

Bcl-x. The alternative use of two 5′ splice sites produces the pro- and anti-apoptotic isoforms of Bcl-x (Fig. 4c). Several regulatory elements have been identified in the regions flanking these splice sites. Intronic regulatory elements (IRE) may repress the use of the 5′ splice site of Bcl-x$_L$ following induction by IL-6 and GM-CSF, or treatment with TPA [128]. In addition, two exonic regions known as CRCE1 and CRCE2 mediate the pro-apoptotic effect conferred by ceramide on the splicing of Bcl-x [129]. Given that the U2 snRNP-associated protein SAP155 binds to CRCE1 and that its genetic depletion by RNA interference also promotes Bcl-x$_S$ usage, the signaling pathway activated by ceramide possibly prevents SAP155 binding. More recently, a role for Sam68 has also been uncovered [130]. Although the *cis*-acting element mediating this function is currently unknown, Sam68 appears to interact with hnRNP A1 to elicit a splicing shift toward Bcl-x$_S$. Three other regions have been identified as important for controlling the use of the alternative 5′ splice sites. One of them (B2) is specifically bound by hnRNP F/H proteins and is required to enforce the use of Bcl-x$_S$ 5′ splice site [131]. The B3 region is bound by SRp30c and enforces Bcl-x$_L$ usage [132]. Finally, a region (SB1) upstream of the Bcl-x$_S$ 5′ splice site distinct from CRCE1 represses the use of the 5′ splice site of Bcl-x$_S$. This SB1-dependent splicing repression is lifted by drugs that inactivate protein kinase C, as well as by a variety of anticancer agents [133, 134]. The pathways that control this aspect of Bcl-x$_S$ splicing vary considerably between different cancer cell lines.

Caspase 2. Members of the SR and hnRNP family of proteins participate in the alternative splicing control of caspase 2 [135]. Overexpressing SR proteins enhances the exclusion of exon 9 to favor the production of the pro-apoptotic isoform Casp2L. In contrast, overexpressing hnRNP A1 stimulates the inclusion of exon 9 and the production of the anti-apoptotic isoform Casp2S [136]. The intronic sequence In100 is a control element modulating the alternative splicing of caspase 2 (Fig. 4d). This element is bound by hnRNP I/PTB and it prevents splicing between exons 9 and 10 through formation of a nonproductive complex with splicing factors recruited at the In100 site [137].

4.2.3   Other Perturbations that Impact Alternative Splicing

As discussed above, cancer cells can accumulate genetic changes that directly affect the splicing machinery or splicing regulatory elements in pre-mRNAs to deregulate alternative splicing patterns. In addition, at least four other molecular processes may affect the production of splicing variants to impact on carcinogenesis. These include transcription, RNA editing, NMD, and signal transduction events.

Transcription

Simple overexpression of pre-mRNAs may contribute to deregulated alternative splicing. For overexpressed pre-mRNAs, there might not be enough available control factors to modulate their splicing. In addition, the sequestration of splicing factors by this pre-mRNA may affect the splicing of other pre-mRNAs, similar to the situation occurring when triplet repeat expansion in the $3'$ untranslated region of the DMPK pre-mRNA sequesters MBNL with an impact on the alternative splicing of cardiac troponin T, tau, insulin receptor, ClC-1, and myotubularin-related 1 pre-mRNAs [138, 139].

The mechanistic coupling of RNA synthesis by RNA polymerase II to alternative splice site choice has been documented [140, 141]. Both enhancer and core promoter elements can modulate the alternative splicing of their nascent pre-mRNA [142, 143]. Promoter identity can control alternative splicing through the differential recruitment of transcription factors and cofactors, such as Brm [124], CoAA [144] and Spi-1 [145], or by recruitment of specific splicing factors, such as SRp20 [146]. Ultimately, promoter-specific transcription complexes can dictate alternative splice site choice by altering the rate of elongation and/or the processivity of RNA polymerase II [147, 148], or through physical interactions with the splicing machinery. Consequently, factors that modify the status of chromatin that change the intrinsic speed of the transcriptional complex or that modify its sensitivity to pausing sites can potentially affect alternative splicing.

In addition to the impact of different complexes at a single promoter on alternative splicing, there is now convincing evidence for the impact of multiple promoters of a single gene on alternative splicing. For Bcl-x [149] and caspase-2 [150], the choice of alternative promoters can define the ratio of splice variant expression. The literature now contains a growing list of genes that contain alternative promoters [151]. In fact, a recent bioinformatics analysis estimated that 52 % of human genes contain

alternative promoters [152]. In this context, oncogenic alterations of transcription factors, as well as genetic or epigenetic changes in promoter regions could influence the alternative splicing patterns in tumor cells. We already know that there must be considerable tissue- and cancer-specific variations in the combinatorial assemblies at promoters. What remains to be discovered is the extent of the contribution of these transcriptional aspects to global patterns of alternative splicing in cancer.

RNA Editing

RNA editing is a process that directly modifies specific adenosine residues into inosines [153]. RNA editing can influence the secondary structure of specific pre-mRNAs in preparation for splicing. In some types of tumors, editing defects can affect the alternative splicing of the PTPN6 phosphatase and glutamate receptor pre-mRNAs [154, 155]. Because the contribution of RNA editing to the alternative splicing of cancer-related genes has not been investigated systematically, it is likely that more examples of this type exist.

NMD

NMD is a surveillance process that eliminates mRNA molecules containing a stop codon localized more than 50 nucleotides upstream of exon/exon junction [156, 157]. Therefore, NMD can potentially neutralize the impact of mutations that create new termination codons [157, 158]. While NMD can affect the accumulation of some splicing variants, its widespread contribution in controlling the expression of splicing isoforms is unlikely [15] but controversial [159]. Interestingly, the non-productive isoforms of splicing factors that are generated from the alternative splicing of exons enriched in ultra conserved sequences form a distinct class of NMD-regulated transcripts [17, 19].

It is possible but yet undocumented that changes in the expression or activity of factors involved in regulation of the NMD may promote the accumulation of the mutated or aberrantly spliced transcripts and therefore may contribute to cancer. Although the number of examples supporting a role for NMD in cancer remains small [160, 161], this pathway appears to contribute to the elimination of mRNA encoding proteins involved in drug-resistance [60]. Such alterations in the NMD pathway may contribute to tumorigenesis.

Signal Transduction

Cell signaling impacts alternative splicing [162, 163] and a typical means by which such changes can be mediated is by the phosphorylation of SR proteins, which causes them to accumulate in different subcellular compartments [164]. The upregulation of PKA and the $MKK_{3/6}$/p38 signaling pathway results in the cytoplasmic localization of hnRNP I/PTB and hnRNP A1, respectively [165, 166]. Another splicing factor displaying this behavior is KSRP, which is implicated in the inclusion a specific exon in the c-src mRNA in neurons [167]. KSRP accumulates in the nucleus when neuroblastoma cells are induced to differentiate [167]. Therefore, the altered subcellular localization of splicing factors via their post-translational modification can potentially play a role in the deregulation of

splicing in cancer cells. The impact of splicing factor localization on the cancer phenotype is likely underappreciated, as documenting changes in localization is considerably more laborious than determining changes in total expression levels of the mRNA or protein.

The interface between signal transduction and splice site selection is just beginning to be explored. The Ras/PI 3-kinase/AKT pathway is often activated in human cancer [168]. This pathway can modulate the activity of SR proteins to affect the alternative splicing of fibronectin and PKC βII [169, 170]. The Ras-dependent signaling pathway is also implicated in the alternative splicing of CD44 [171]. The intricate interplay between alternative splicing and cell signaling is illustrated by the fact that just as signaling affects alternative splicing, so alternative splicing can affect signaling. For example, the inclusion of exon v6 in CD44 promoted by Ras helps to sustain late Ras signaling [172]. The SR protein kinase SRPK1 is overexpressed in many types of cancers and its downregulation increases the sensitivity of cells to anticancer drugs [173], and MAP2 K is alternatively spliced in response to activation of SRPK1 [174]. Another recent example of a link between signaling and splicing control involves the Notch3 signaling pathway which regulates the splicing of Ikaros in leukemia by inducing the RNA-binding protein HuD [175–177]. The deregulated expression of CDK12 and cyclin L1/L2 can also affect alternative splicing decisions [178].

Thus, it would not be too surprising if cancer-specific alterations in signaling pathways impact the production of isoforms that contribute to neoplastic transformation. However, the contribution of signal transduction events to splicing decisions that are relevant to cancer remains to be more fully investigated.

Arginine methylation of splicing factors also affects their localization and activity [179, 180]. Recently, the arginine methylase CARM1 was shown to control splicing decisions [181]. Although cancer-specific defects in the arginine methylation of splicing factors are yet to be reported, important contributions of this pathway to various aspects of cell growth can be anticipated.

5 Outlooks and Challenges

5.1 Global Detection of Splicing Variation in Cancer

The existence of cancer-specific signatures made up of individual alternative splicing events was initially investigated by using large-scale compilation of cDNAs and expressed-sequences tags (ESTs). Computational approaches revealed considerable differences between the alternative splicing patterns of normal and cancer prostate tissues [182, 183]. Genome-wide profiling of splicing events became possible with the development of DNA oligonucleotide microarrays. Using this technology, one of the first observations was that the genes displaying tissue-specific alternative splicing were largely different from the genes presenting tissue-specific differences in steady-state expression levels [184]. Indeed, a quarter of the genes that showed alternative splicing between normal and cancerous

prostate had no detectable change in overall gene expression level [185]. A similar conclusion was obtained when mining for differences in expression profiles and the detection of splicing isoforms between melanoma and melanocytes [186]. Overall, the classification of tumors was improved when alternative splicing was considered, strongly arguing in favor of mining the wealth of alternative splicing diversity for the purpose of developing a complementary disease-specific signature that could have diagnostic and prognostic value.

Splicing-sensitive arrays were generated using limited sets of alternative splice junction probes and used to detect splicing changes in cancer tissues. For example, changes in splicing profiles and the abundance of splicing factors in Hodgkin lymphoma was observed using an array that measured mRNA levels and 100 splicing events [187]. More recently, a similar design was used to compare alternative splicing of 64 genes in breast cancer cell lines and xenografts [188]. In this case, four genes were found to be differentially spliced between breast cancer and normal cell lines and four other tumor-associated genes were spliced differently according to cell culture conditions.

A new technique called DASL was developed to measure the expression of cancer-related splice isoforms. This technique combines targeted microarray and PCR techniques and treats splicing isoforms as separate genes. DASL therefore estimates changes in global gene expression level, as a normal microarray does, while providing additional information on the ratio of splice isoforms variants [189]. Recently, this approach has been used for the analysis of 1,500 different splice variants from 364 genes in six prostate tumor cell lines and 22 prostate tumors [185, 190]. Fourteen genes had different isoforms whose expression in normal tissues inversely correlated with their expression in tumor samples, implying a likely switch in alternative splicing.

Companies have also designed microarrays for cancer biomarker discovery using known alternatively spliced junction probes identified through bioinformatics mining of EST libraries. The Jivan cancer-specific splice variant array covers 524 putative cancer-specific splices although this has been largely superseded by their total splice form microarray that includes 193,000 specific splice junctions. ExonHit also has pathway-specific microarrays and a 'genome-wide' alternative splice junction microarray. The other approach to splicing discovery is to print probes for every exon:exon junction and deduce alternative splicing patterns from the relative junction expression patterns. The first large-scale splicing microarrays using this approach covered about half of the known exon:exon junctions in the human transcriptome and monitored splicing globally in multiple normal and cancer cell lines [191]. Affymetrix now produces a Human Exon GeneChip microarray that detects over 1,000,000 different human exons. This was used to compare splicing between ten matched normal tumor colon cancer pairs and nine high confidence differences were confirmed by RT-PCR [192].

One drawback of most current global approaches is that they rely on previously documented splicing events and are therefore not designed to discover novel splicing events. Because our current collection of existing splicing events is likely

incomplete, biased, and poorly validated, there is a need to incorporate strategies that can identify novel splicing events and technologies that can accurately validate the quantitative differences detected between samples. Current microarray technologies may be superseded in the future by high-throughput sequencing technologies of single molecules [193]. However, although all the above approaches are designed to improve the annotation and assessment of alternative splicing events, they all fail to provide a description of the complete structure of splice isoforms. Indeed, pre-mRNA often sustains multiple alternative splicing events, sometimes involving regions that are far apart. Given that splicing decisions taken in one region can affect the splicing outcome of an apparently distinct unit [194], we cannot assume that all combinations of potential isoforms are represented in the mRNA population. The only currently reliable way to obtain this information remains through cDNA cloning and large-scale projects in this direction would provide a useful complement to current high-throughput mapping efforts directed at specific alternative splicing units.

5.2   Depleting Specific Splice Isoforms

We have seen that mutations in splicing elements of key genes and expression defects of splicing control factors can contribute to neoplastic transformation. The alterations in the splicing profiles observed in tumors may lead to the production of novel isoforms. In other cases, the isoforms may correspond to molecules that are normally expressed in other cell types or at other stages of development. Most frequently, splicing alterations will involve differences in the relative abundance of isoforms already expressed in the normal tissue. Although several of these alterations may have a neutral impact, other splicing alterations may confer growth advantages to cancer cells. For example, the signaling pathways that inhibit cell proliferation or induce cell death may be neutralized leading to cell propagation and invasion. Considering the enormous potential for diversity emanating from alternative splicing, the number of isoforms that are known to affect the growth of cancer cells is most probably vastly underestimated. More extensive annotation of the function of different isoforms is a priority that represents a challenge both in terms of commitment and methodology.

To help with the goal of attributing function to splice variants, the RNA interference (RNAi) technology represents a useful approach because it can be used to reduce the expression of specific isoforms. Although this approach has been used successfully in *Drosophila* cells [195], it is yet to be used in a systematic manner in human cells. If RNA interference-based approaches turn out to promote transcriptional silencing in addition to RNA degradation [196, 197], depletion of specific mRNA isoforms may only be possible with siRNAs targeting exon-exon junctions. This caveat could seriously compromise the use of RNA interference as a tool to assess isoform function.

5.3   Reprogramming Alternative Splicing

A different approach aims at reprogramming alternative splicing decisions. The spectacular physiological improvement obtained by reprogramming the Duchenne muscular dystrophy gene in a mouse model [198] supports applying this strategy to cancer. The original version of the approach employs a complementary oligonucleotide to cover the targeted splice site (Fig. 5a). By blocking the 5′ splice site of the anti-apoptotic Bcl-x$_L$ with 2′-O-methyl oligonucleotides, Mercatante et al. [199]
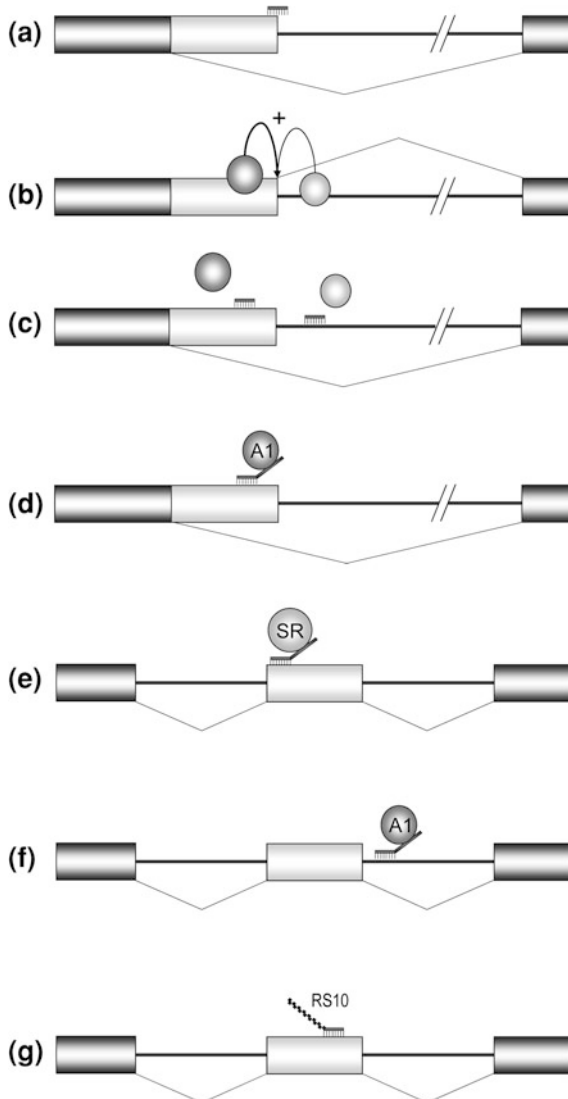
◄**Fig. 5** Reprogramming of alternative splicing through use of oligonucleotides. **a** An oligonucleotide blocking a splice site favors the use of an alternative site. **b** Oligonucleotides complementary to exonic or intronic controlling elements can prevent the binding of control factors. If these elements are ESEs and ISEs as shown, the oligos will stimulate splicing to an alternative site. **c** A complex between hnRNP A1 protein and the tail of an oligonucleotide partially complementary to an exonic sequence can provoke steric interference to reduce the use of a splice site and favor alternative splice site selection. **d** When the tail of the oligo contains a sequence of high affinity for a SR protein, splicing to the adjacent site can be stimulated. **e** If the hnRNP A1 binding tail is contained in an oligo complementary to an intron region near the 5′ splice site, splicing stimulation can occur. **f** A PNA (*peptide-nucleic acid*) portion covalently linked to an RNA sequence complementary to an exon can stimulate exon inclusion when the PNA tail contains repetitions of the dipeptide arginine-serine to mimic the RS domain of SR proteins

could decrease the concentrations of the Bcl-x_L isoform and increase the level of the corresponding pro-apoptotic Bcl-x_S isoform. This change in the splicing profile of Bcl-x increased the sensitivity of cells to chemotherapeutic agents and even induced apoptosis in some cell lines [200]. Similar approaches have been successfully applied on the FGFR1 and the MYC pre-mRNAs [201, 202].

An alternative strategy involves using antisense oligonucleotides that target splicing control elements (Fig. 5b). An oligonucleotide complementary to a sequence upstream of 5′ splice site of Bcl-x_L strongly repressed the use of this site [203] probably because its hybridization neutralizes a positive controlling element [131]. In a similar way, inactivating intronic repressor elements can also be used to modulate alternative splicing [202, 204]. Modification of the oligonucleotide with a non-hybridizing tail that contains one or several binding sites for a protein produces a bifunctional oligonucleotide that can recruit factors to a specific region on the pre-mRNA. For example, binding of hnRNP A1 to the tail of a bifunctional oligonucleotide sterically interferes with the efficient utilization of a neighboring 5′ splice site (Fig. 5c) [205]. The architecture of the tail can be modified to provide binding sites for other factors [206]. The general concept is flexible and changing the identity of the recruited protein or modifying the hybridization site can be used to stimulate splicing (Fig. 5d and e) [72, 207]. A PNA (peptide-nucleic acid) version has also been used with success. In this case, the tail is made of alternating serine and arginine amino acids that mimic SR proteins in their ability to promote exon inclusion (Fig. 5f) [208, 209]. The above approaches can be used to document the functions of different splice isoforms and therefore help to define new therapeutic targets. The development of oligonucleotides whose chemistries are compatible with human use may offer new therapeutic means for anticancer treatment. Although *trans*-acting non-coding nucleic acid molecules to reprogram splicing have been used by researchers for many years, only recently did we realize that this molecular strategy is used normally by cells to modulate splicing decisions. A snoRNA was recently shown to shift the splicing of a serotonin receptor [210]. Likewise, microRNAs (miRNAs) have very recently been shown to regulate alternative splicing during muscle differentiation [211, 212]. Given that miRNA expression is often altered in cancer cells [213], it is likely that additional examples of misregulation of splicing through miRNAs will be found in cancer-associated genes.

Another interesting approach consists in modifying the expression or activity of proteins that affect alternative splicing. This strategy has potential value given that the inhibition of the SRPK1 kinase through RNA interference reduces cellular proliferation and increases apoptosis in cells derived from pancreatic tumors [173, 174]. A more classic pharmacological approach consists in screening libraries for chemical compounds to identify small antagonist molecules with therapeutic value. An application of this strategy has resulted in the discovery of compounds that inhibit other kinases specific for SR proteins and that affect alternative splicing [214, 215]. Recently, screening assays have uncovered molecules that directly target SR proteins and that preferentially inhibit splicing events required for HIV replication [216]. These encouraging results justify that similar approaches be attempted with other regulatory splicing factors.

6   Conclusions

Cancer can arise from alterations in a variety of cellular pathways including signal transduction, cell cycle, and apoptosis. Alternative splicing is an important process that participates in the complex regulation of these cancer-related pathways. Despite incomplete information, it is becoming increasingly evident that defects in alternative splicing imposed by mutations or changes in the levels of splicing factors can generate isoforms whose activities contribute to the initiation and progression of cancer. Given current efforts at documenting the function of splice variants and at cataloguing cancer-specific splicing alterations, we can expect that the role of alternative splicing in cancer will provide many novel anticancer targets. In the meantime, the results of different approaches aimed at reprogramming splice site usage will pave the way for novel therapeutic strategies against cancer.

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