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Abnormal alternative splicing promotes tumor resistance in targeted therapy and immunotherapy

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ABSTRACT

Abnormally alternative splicing events are common hallmark of diverse types of cancers. Splicing variants with aberrant functions play an important role in cancer development. Most importantly, a growing body of evidence has supported that alternative splicing might play a significant role in the therapeutic resistance of tumors. Targeted therapy and immunotherapy are the future directions of tumor therapy; however, the loss of antigen targets on the tumor cells surface and alterations in drug efficacy have resulted in the failure of targeted therapy and immunotherapy. Interestingly, abnormal alternative splicing, as a strategy to regulate gene expression, is reportedly involved in the reprogramming of cell signaling pathways and epitopes on the tumor cell surface by changing splicing patterns of genes, thus rendering tumors resisted to targeted therapy and immunotherapy. Accordingly, increased knowledge regarding abnormal alternative splicing in tumors may help predict therapeutic resistance during targeted therapy and immunotherapy and lead to novel therapeutic approaches in cancer. Herein, we provide a brief synopsis of abnormal alternative splicing events in cancer progression and therapeutic resistance.

Introduction

According to the Human Genome Project, the human genome contains approximately 23,000 genes, which is far from the diversity of the proteome encoded by genes. In 1980, it was first revealed that a gene could be transcribed into two different mRNAs [1]. Alternative splicing is the mechanism that processes pre-mRNA through different splicing modes to produce a variety of mature mRNAs with different structures and functions (**Fig. 1**). Reportedly, 95% of human gene expression is regulated by alternative splicing [2]. Alternative splicing greatly improves the complexity of the transcriptome and the diversity of the proteome as a significant regulatory mechanism of gene expression. If the alternative splicing pathway goes unchecked, it can lead to protein expression disorders and various diseases, including cancer, neurodegenerative disorders, muscular dystrophies, and cardiovascular and immunologic diseases [3-5]. In particular, hundreds of splicing events are reportedly disordered in cancer [6]. These abnormal splicing events are associated with malignant progression and therapy resistance in tumors [7]. Specifically, tumor cells can express abnormal proteins that promote cancer progression through abnormal splicing events. Additionally, abnormal splicing variants develop resistance by mediating the loss of antigen targets or changing the target of drug action in cancer. This review focuses on recent research progress concerning alternative splicing, which promotes tumor malignant progression and develops resistance to targeted therapy and immunotherapy.

The physiological process of alternative splicing

The alternative splicing process is carried out by spliceosomes and splicing factors (**Fig. 2**). First, the arginine/serine-rich domain (located at the C-terminal) of SRSF proteins is phosphorylated by Serine/arginine-rich protein-specific kinase (SRPKs) in the cytoplasm, which are transported to the nucleus by transportin-SR (TRN-SR) [8]. Then, phosphorylated SRSF proteins are transported to the nuclear speckle domains and hyper-phosphorylated by cdc2-like kinase 1 (CLK1). Hyper-phosphorylated SRSF proteins can bind pre-mRNA via an RNA recognition motif (RRM, in the N-terminal) [9]. U1 small nuclear ribonucleoprotein (snRNP) and U2 snRNP are recruited to combine with the splice site of the intron. U1 snRNP binds the conserved sequence G-U of the 5′ splice site, and U2 snRNP replaces the branch-site binding protein (BBP) and binds to the conserved sequence A-G of the 3′ splice site [10]. Then, U1 snRNP interacts with U2 snRNP to form the spliceosome A. Second, U4, U6, and U5 snRNPs are assembled into tri-snRNP under the action of phosphorylated pre-mRNA processing factor kinase 31

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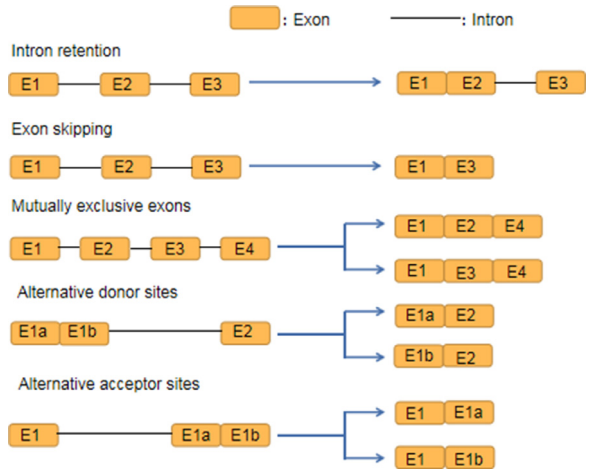

Fig. 1. The modes of pre-mRNA alternative splicing. Intron retention: the intron sequence is retained in the mature mRNA. Exon skipping: a single entire exon sequence is removed. Mutually exclusive exons: one of the adjacent exons is randomly removed or kept. Alternative donor sites: a different donor splice site is used for removing or keeping part of exon sequence. Alternative acceptor site: a different acceptor splice site is used for removing or keeping part of exon sequence.

(PRP31) and pre-mRNA processing factor kinase 6 (PRP6), both phosphorylated by pre-mRNA processing factor kinase 4k (PRP4k) [11,12]. The tri-snRNP interacts with spliceosome A through the pre-mRNA processing factor kinase 28 (PRP28), which is phosphorylated by SRPK2. The U2 snRNP displaces U4 snRNP to combine with U6 and U5 snRNPs, U6 snRNP replaces U1 snRNP to bind the conserved sequence G-U of the 5′splice site of the intron, resulting in the conformation of spliceosome B [8]. Then, the pre-mRNA is required to undergo two transesterification reactions for splicing into mature mRNA. In the first transesterification reaction, the 2′ hydroxyl of adenylate located at the 3′ splice site of the intron performs a nucleophilic attack on the phosphodiester bond in the 5′ splicing site, breaking the phosphodiester bond between exons and introns, thereby generating a 2′–5′ phosphodiester bond and resulting in the conformation of the intron's lariat structure. Thus, spliceosome B transforms into spliceosome C. Following the second transesterification reaction, the newly generated free hydroxyl in the exon 3′ splicing site performs a nucleophilic attack on the phosphodiester bond in the 3′splice site of the intron, thereby completely breaking the connection between the intron and exon; consequently, the intron lariat is released and degraded [13]. The adjacent exons are then pulled together and connected via U5 snRNP, which requires GTP depletion. The remaining U2, U5, and U6 snRNPs are released from spliceosome C to be utilized in subsequent rounds of alternative splicing. Subsequently, the hyperphosphorylated SRSF proteins are dephosphorylated under the action of protein phosphatase 2A (PP2A) or protein phosphatase1 (PP1) and released into the cytoplasm along with the mRNA for reuse. Thus, the pre-mRNA undergoes a series of processes and is finally transformed into mature mRNA.

Alternative splicing promotes cancer malignant progression

Oncogenesis is a complex multistep process that involves the abnormal expression of several genes. In cancer cells, abnormal expression of splicing factors leads to disordered alternative splicing events, resulting in abnormal protein expression and promoting malignant progression (Table 1).

RBM5, as a tumor suppressor gene and splicing factor, improves the production of meaningless mRNAs by recognizing incorrect 3′splice sites of epidermal growth factor receptor (EGFR) pre-mRNA, thereby inhibiting the proliferation of tumor cells [14]. However, the downregulation of RBM5 has been observed in various tumors, consequently upregulating the expression of EGFR and promoting tumor cell proliferation

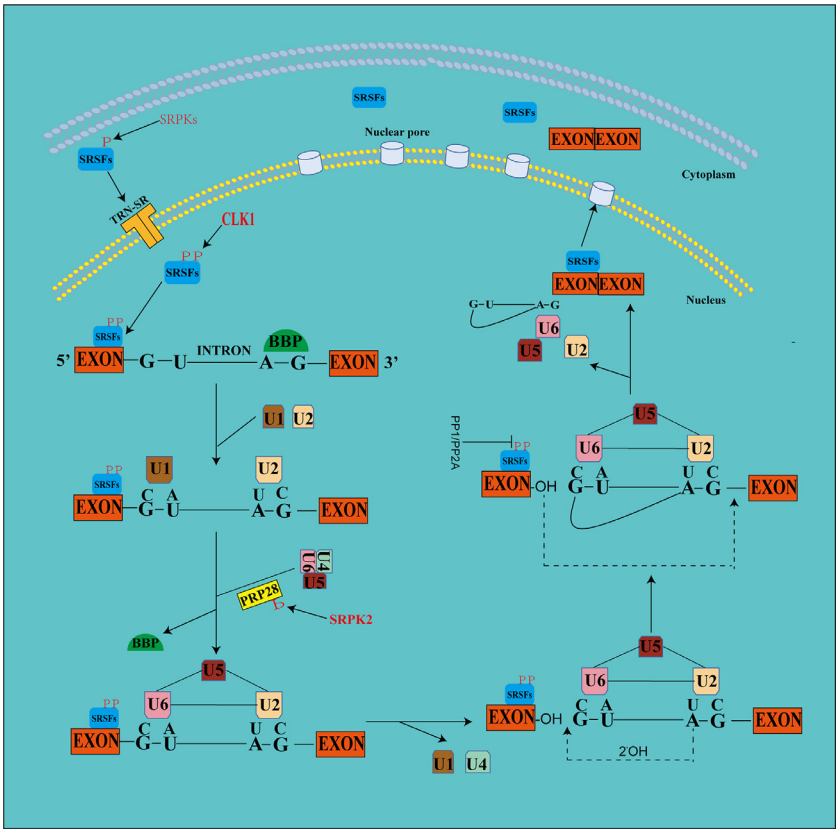

Fig. 2. The physiological process of Pre-mRNA Alternative Splicing. The whole process depends on spliceosome and splice kinase. Please refer to the text for specific examples and references.

Table 1
Abnormal alternative splicing variants promotes cancer malignant progression.

Gene	splice variant	Role	expression in cancer	AS event	Splicing factors	Ref
Mnk2	Mnk2-a	Pro-apoptotic	Low	Exon skipping	Overexpressed SRSF1	[18]
	Mnk2-b	Pro-proliferative	High			
BCL-X	BCL-XL	Anti-apoptotic	High	Alternative donor sites	Overexpressed SRSF1 and SRSF9	[20]
MCL	MCL-1L	Anti-apoptotic	High	Exon skipping	Overexpressed hnRNP K and hnRNP F/H	[21]
CD44	CD44s	Pro-invasive	High	Exon skipping	Down-regulated PCBP1	[22,23]
VEGF-A	VEGF-Axxxa	Pro-angiogenic	High	Alternative acceptor sites	Overexpressed SRSF1, SRSF5 and SRPK1	[26]
	VEGFAxxxb	Anti-angiogenic	Low			
PKM	PKM2	Promote aerobic glycolysis	High	Mutually exclusive exons	Overexpressed SRSF3, hnRNPA1 and PTBP1	[31]

and distant metastasis [15-17]. Mnk2 has two transcripts, the full-length transcript Mnk2-a and exon 13a skipping transcript Mnk2-b (lack of MAPK domain). Mnk2-a can directly interact with p38a-MAPK through the mitogen-activated protein kinase (MAPK) domain, thereby inducing apoptosis. Reportedly, overexpressed SRSF1 could switch the Mnk2 splicing to the Mnk2-b transcript in cancer, which promoted cell proliferation [18]. Conversely, members of the BCL2 family are key regulators of apoptosis, and the anti-apoptotic members Bcl-xL and MCL-1 L protect cells from apoptotic death [19]. Overexpression of SRSF1 and SRSF9 contributes to the increased production of BCL-XL transcripts in cancer cells, which is an anti-apoptotic splicing variant of the BCL-X gene [20]. Similarly, it has been shown that overexpressed splicing factors hnRNP K and hnRNP F/H could promote the switch in MCL pre-mRNA splicing toward MCL-1 L transcript (an anti-apoptotic isoform) in breast and ovarian cancers [21].

CD44 is a glycoprotein on the cell membrane, a widely recognized stem cell marker in tumors. Alternative splicing transcribes CD44 to the mesenchymal spliced variant (CD44s) and epithelial spliced variants (CD44v). Interestingly, transforming growth factor (TGF)-β promotes the switch from CD44 isoform to CD44s by inducing the degradation of poly c binding protein 1 (PCBP1) in carcinoma cells, resulting in the overexpression of CD44s in the process of epithelial-mesenchymal transition (EMT) induced by TGF-β [22,23].

Vascular endothelial growth factor (VEGF) is an essential growth factor that promotes proliferation of vascular endothelial cells. The VEGF-A pre-mRNA encodes two splicing variants by recognizing different splicing sites, pro-angiogenic VEGF-Axxxa and anti-angiogenic VEGF-Axxxb isoforms [24]. Notably, the ratio of VEGF-A165b/VEGFA165a expression was downregulated in numerous solid tumors [25]. Reportedly, the overexpression of SRSF1, SRSF5, and SRPK1 is related to the high expression of VEGF-Axxxa isoforms in pharyngeal tumors [26].

The pyruvate kinase M gene encodes two splice variants by removing exon 9 or exon 10, which are PKM1(lack of exon 10) and PKM2 (lack of exon 9) [27]. Under hypoxic conditions, tumor cells mainly rely on aerobic glycolysis to supply energy [28]. Interestingly, PKM2 facilitates the metabolic transformation of tumor cells [29]. It was reported that SRSF3, hnRNPA1, and polypyrimidine tract-binding protein 1 (PTBP1, also termed hnRPN1) jointly induce the expression of the PKM2 isoform during cancer [30]. SRSF3 induces the retention of exon 10 by binding to exonic splicing enhancer (ESE) sequences during PKM pre-mRNA splicing. Then, PTBP1 crosslinks to the pyrimidine tracts within the 3′ splice sites of exon10, which inhibits other splicing factors from identifying exon 10, thus preventing the exclusion of exon10 [31]. Conversely, PTBP1 promotes exon 9 exclusion by binding to the splice sites flanking exon 9.

Alternative splicing promotes the development of tumor resistance during targeted therapy

An important basis for tumorigenesis is the reprogramming of the gene expression profile, which results in the production of proteins that are beneficial for tumor cell growth and proliferation [32]. These proteins are differentially expressed between normal cells and tumor cells and can be designed as specific targets for anticancer drugs, which results in the elimination of tumor cells while reducing their cytotoxicity to normal cells [33]. However, the inevitable drug resistance of tumor cells considerably limits the clinical application of targeted drugs. Abnormal alternative splicing is the main factor altering the gene expression profiles of tumor cells, which induces resistance by changing the target point and signal transduction pathway of targeted drugs (Table 2).

Alternative splicing promotes resistance to targeted drug by changing the target of drug action

Imatinib is an anti-tumor drug that inhibits tyrosine kinase, frequently employed in the treatment of chronic myelogenous leukemia. Imatinib can induce the inactivation of BIM protein (a member of the BCL2 family) by binding to its BH3 domain, thereby promoting apoptosis in leukemic cells [34]. However, BIM-ϒ, a spliced variant of BIM that skips exon 4, is reportedly involved in leukemic cell resistance to imatinib [35]. BIM may lack the target of imatinib due to the lack of exon4, which further induces resistance to imatinib. Similarly, enzalutamide is a targeted drug commonly used to treat prostate cancer, targeting the ligand-binding domain (LBD) of the androgen receptor (AR), thereby blocking the interaction between androgen and AR. However, it has been shown that hnRNPA1 could induce the generation of ARv7, a splicing variant of AR. The ARv7 transcript lacks a cryptic exon called CE3, which encodes LBD. Thus, the Arv7 protein lacks LBD, and enzalutamide lacks a target in prostate cancer; this further promotes prostate cancer cell resistance to enzalutamide [36,37].

CD20 and CD19, as specific biomarkers, are widely expressed in malignant B cells and are used as targets for treating B-cell lymphoma. Rituximab, ofatumumab, and obinutuzumab have been successfully used to treat B-cell lymphoma by targeting CD20; however, acquired drug resistance is considered a major obstacle to their clinical application. A recent study has reported that five new splice variants of CD20 (D177-CD20, D393-CD20, D480-CD20, D618-CD20, and D657-CD20) have been identified in malignant B cells, which are produced by exon skipping or alternative splice sites [38]. Most importantly, it has been proven that D393-CD20 is involved in resistance to rituximab. Part of exon 3 to part of exon 7 is spliced in the D393-CD20 transcript, resulting in its code protein lacking the rituximab recognition epitope, indicating that this truncated D393-CD20 protein develops resistance to rituximab [39]. Similarly, the D177-CD20 and D480-CD20 proteins also lack the ofatumumab recognition epitope, whereas the D657-CD20 and D618-CD20 proteins lack the obinutuzumab recognition epitope [38]. However, it remains to be determined whether the lack of these epitopes is associated with resistance toward ofatumumab and obinutuzumab. Thus, we assume that the four splice variants may develop resistance to ofatumumab and obinutuzumab according to the mechanism of D393-CD20 protein in drug resistance. An identical mechanism was also recognized in the resistance to blinatumomab. Blinatumomab is used to treat B-cell acute lymphoblastic leukemia, which induces a T-cell-mediated immune response to cancer cells by directing CD3-positive T cells to tar-

Table 2
Abnormal alternative splicing events promotes tumor resistance to targeted therapy.

Gene	splice variant	AS event	Role	Mechanism	Ref
BIM	BIM-ϒ	Exon 4 skipping	Resistance to Imatinib	Missing target point of drug	[35]
AR	ARv7	Exon CE3 skipping	Resistance to Enzalutamide	Missing target point of drug	[36,37]
CD20	D393-CD20	Exon 3–7 skipping	Resistance to Rituximab	Missing target point of drug	[39]
CD19	CD19 ex2part	Part exon 2 skipping	Resistance to blinatumomab	Missing target point of drug	[40]
	CD19 Δex5–6	Exon 5–6 skipping	Resistance to blinatumomab	Secreted extracellularly, worked as a decoy to bind drug	[41]
RAF	p61 BRAFV600E	Exons 4–8 skipping	Resistance to Vemurafenib	Reactivate MAPK/ERK pathway	[52,53]
ERα	ERα36	Exons 7–8 skipping	Resistance to Tamoxifen	Activate MAPK/ERK pathway Activate Sphk1/S1p axis	[56]

get CD19-positive malignant B cells. Reportedly, CD19 has four splice variants: the full-length transcript, exon 2 skipped transcript (termed CD19 Δex2), part exon 2 spliced transcript (termed CD19 ex2part), and exon 5–6 skipped transcript (termed CD19 Δex5–6). Interestingly, the expression levels of CD19 ex2part in malignant B cells resistant to blinatumomab were significantly higher than in those sensitive to blinatumomab, and the CD19 ex2part was higher in the recurrence sample after treatment than in the sample before treatment during blinatumomab therapy. The CD19 ex2part protein lacks residues Met1-Leu151, which is the domain recognized by blinatumomab [40], suggesting that CD19 exon 2 encodes the epitope targeted by blinatumomab; therefore, CD19 ex2part and CD19 Δex2 may both be involved in resistance to blinatumomab. Then, exons 5 and 6 encode the transmembrane and cytosolic domains of the CD19 protein, promoting the secretion of CD19 Δex5–6 protein. The CD19 Δex5–6 variant served as a decoy combined with blinatumomab in the tumor microenvironment, thereby inducing resistance [41].

Trastuzumab, a monoclonal antibody targeting HER-2, is commonly used to treat HER2-positive aggressive breast cancer. However, approximately 50% of patients with HER2-positive breast cancer maintain a low response to trastuzumab [42]. It was argued that HER2Δex16, a splice variant of HER-2, might mediate the resistance to trastuzumab. However, this theory remains controversial. On the one hand, the HER2Δex16 splice variant lacks 16 amino acids encoded by exon16, which may impair the interaction with trastuzumab. In vitro experiments, the wild-type HER2-expressing cell line showed significant sensitivity to trastuzumab, while the HER2Δex16-expressing cell line was resistant to trastuzumab. Interestingly, it was indicated that trastuzumab similarly combined the wild-type HER2 and HER2Δex16 at the cell surface [43]. Therefore, it is suggested that the HER2Δex16 variant also has an epitope recognized by trastuzumab. Additionally, another study revealed the mechanism underlying HER2Δex16 resistance toward trastuzumab. Reportedly, HER2Δex16 is a cell surface receptor that is inefficiently internalized; this results in the transmission of drug signals to be blocked, inducing resistance to trastuzumab [44]. In contrast to in vitro studies that revealed trastuzumab resistance in HER2Δex16 expressing cells, trastuzumab significantly inhibited the growth of HER2Δex16 overexpression in MCF10A cell-derived xenograft tumors in vivo [45]. This could be attributed to differences in physiological conditions in vivo and in vitro that resulted in these two sharply contrasting perspectives. It has been shown that aberrant vasculature dramatically alters the tumor microenvironment, promoting the malignant progression of cancer and inhibiting the response to anticancer therapies [46]. In solid tumors, this abnormal vascular system supports the hypoxic characteristics of the tumor microenvironment. Hypoxia induces the stable expression of the transcription factor hypoxia-inducible factor 1α (HIF1α), which enhances the malignant performance of cancer cells [47,48]. Conversely, hypoxia is an important factor that induces tumor cell resistance to anticancer therapies, including conventional radiotherapy and cytotoxicity. More importantly, it was proven that an insufficient blood supply could impair the treatment outcomes observed with monoclonal antibodies in solid tumors [49]. Considerably regular vascularization with numerous endothelium-lined small vessels has been observed in xenograft tumors derived from overexpressing HER2Δex16 cells, compared with those derived from overexpressing wild-type HER2 cells [50]. Thus, we postulate that this difference may be attributed to the complex vascular system in xenograft tumors, and HER2Δex16 may promote the formation of a more regular vascular system in tumors.

Alternative splicing promotes resistance to targeted drugs by changing the signal transduction pathway

BRAF is an integral part of the MAPK pathway responsible for transmitting extracellular signals from RAS to MEK, thereby activating the RAS-RAF-MEK-ERK pathway and promoting tumor cell malignant progression. Vemurafenib inhibits the activity of BRAF by targeting the ATP-binding site, thereby inhibiting BRAF activation by RAS and completing the signal transmission. Thus, vemurafenib is often used to treat melanoma in the BRAF-V600E mutant [51]. However, some patients develop acquired resistance following vemurafenib treatment for a specific period, which is attributed to the recovery of the blocked MAPK pathway. p61 BRAF V600E is a splice variant lacking the RAS binding domain (RBD), encoded by exons 4–8. It has been confirmed that the lack of RBD induces the dimerization and activation of BRAF to proceed in a manner independent of RAS, thereby restoring signal transmission and reactivating the MAPK/ERK pathway [52,53]. Thus, p61 BRAF V600E promotes the development of resistance to vemurafenib by restoring the MAPK signaling pathway.

The estrogen receptor (ER) induces the transcription of target genes in response to estrogen stimulation, which is the main factor associated with breast cancer development. Accordingly, tamoxifen, an anticancer drug targeting the ER, inhibits the proliferation of cancer cells by altering the conformation of estrogen receptors and disturbing the transcription of target genes in breast cancer [54]. However, some patients with breast cancer develop resistance to tamoxifen. The ER family mainly includes two types of members: ERα and ERβ. Estrogen stimulates cell proliferation mainly by activating ERα. ERα has three splice variants: the full-length isoform ERα66 and two truncated isoforms, ERα36 and ERα46. Interestingly, ERα36 has been found to be closely related to tamoxifen resistance. ERα36 transcript skipping exon7 and exon 8 lacks a transcriptional activation domain and is therefore unable to directly activate target gene transcription [55]. However, studies have revealed that ERα36 can activate alternate pathways to mediate the proliferation of tumor cells, including the MAPK/ERK and PI3K/ATK pathways [56]. The ERα36 receptor is responsible for sphingosine kinase 1 (SphK1) activation via estrogen, which in turn enhances sphingosine-1-phosphate (S1P)-mediated activation. SphK1 plays a significant role in the malignant progression of breast cancer [57]. Most importantly, tamoxifen and estrogen are activators of ERα36.

Alternative splicing promotes tumor resistance to immunotherapy

Anti-tumor immunotherapy is the central pillar of future tumor treatment strategies, in which the host-based immune system utilizes immune checkpoint inhibitors or adoptive immune cells to eliminate tu-

Table 3
Abnormal alternative splicing events promotes tumor resistance to immunotherapy.

Gene	splice variant	AS event	Role	Mechanism	Ref
PD-L1	PD-L1 v242	Unclear	Resistance to aPD-L1	Secreted extracellularly, worked as a decoy to bind drug	[63]
	PD-L1 v229	Unclear			
PD-1	PD-1Δex3	Exon3 skipping	Resistance to aPD-1	Secreted extracellularly, worked as a decoy to bind drug	[65]
CTLA-4	sCTLA-4	Exon3 skipping	Resistance to anti-CTLA-4	Secreted extracellularly, worked as a decoy to bind drug	[76]
CD19	CD19 Δex2	Exon2 skipping	Resistance to CART-19	Missing target point of drug	[82]

mor cells. As a result of the memory function of the adaptive immune system, immunotherapy can continuously eliminate tumor cells for a prolonged period, resulting in reduced side effects in patients, with improved long-term survival [58]. Although immunotherapy has several advantages when compared with traditional treatment regimens, it is yet to gain significant popularity in clinical settings. This is attributed to the high resistance of some tumors, and different domains of the same tumor entity have distinct response frequencies to immunotherapy [59]. Reportedly, alternative splicing is involved in tumor-immune interactions by regulating the expression of related genes. Notably, some abnormal alternative splicing events contribute to tumor cell immune escape [60]. More specifically, tumor cells can secrete proteins with abnormal structures through abnormal alternative splicing, which act as a decoy to bind immune checkpoint inhibitors extracellularly. Conversely, abnormal alternative splicing events mediate the loss of tumor cell surface antigens, resulting in the failure of some adaptive immune cells to recognize tumor cells (Table 3).

Alternative splicing promotes tumor resistance to immune checkpoint inhibitors

In tumors, the programmed death-1 (PD-1)/programmed cell death ligand 1 (PD-L1) signaling pathway contributes to the immune escape. PD-1 is mainly expressed on the surface of immune cells, while PD-L1 is expressed on a variety of tumor cells. PD-1 is activated by binding PD-L1. Activated PD-1 then forms negative costimulatory microclusters with T cell receptors (TCRs) by recruiting Src homology 2 domain-containing tyrosine phosphatase 2, which results in dephosphorylation of extracellular regulated protein kinases (ERK), Vav1, and phospholipase Cγ2 (PLCγ2) signaling molecules, leading to the failure of T cell activation [61]. Interestingly, anti-PD-1/PD-L1 antibodies (aPD-1/aPD-L1) have been effectively used to treat diverse cancers; however, some tumor types, such as lung and pancreatic cancers, have shown a low response frequency to these antibodies. Recently, a cancer-derived PD-L1 splice variant was identified in multiple cancers, which could be secreted out of the cell to accumulate in the tumor microenvironment, thus differing from the wild-type PD-L1 expression observed on the surface of tumor cells [62]. Notably, it was proven that the PD-L1 splice variant is involved in aPD-L1 resistance. For example, it was observed that the mutation of TAR DNA binding protein (TDP-53) might influence the alternative splicing process of PD-L1 pre-mRNA, resulting in the formation of two novel PD-L1 splice variants (PD-L1 v242 and PD-L1 v229). PD-L1 v242 and PD-L1 v229 are secreted into the tumor microenvironment owing to the lack of a transmembrane domain. The secreted PD-L1 v242 and PD-L1 v229 splicing variants can afford resistance to PD-L1 blockade immunotherapy by capturing aPD-L1 [63]. However, further studies are needed to clarify how TP53 mediates the alternative splicing process of PD-L1. Similarly, PD-1 has five forms, consisting of four truncated isoforms of exon skipped and the full-length isoform [64]. Reports have confirmed that the truncated PD-1Δex3 is the main splice variant regulated by Matrin 3 (MATR3). The transmembrane domain of PD-1 is encoded by exon3; therefore, PD-1Δex3 is a secreted isoform like PD-L1 v242 and PD-L1 v229 [65]. Consequently, we hypothesized that PD-1Δex3 competitively binds to aPD-1 to induce resistance to PD-1 blockade immunotherapy.

The cytotoxic T-lymphocyte antigen 4 (CTLA-4), an inhibitory receptor, is widely expressed on activated T cells and contributes to immune homeostasis and tolerance [66]. CTLA-4 has two isoforms, the membrane-bound receptor form (mCTLA-4) and the secreted form (sCTLA-4). sCTLA-4 is a splicing variant that lacks exon3, which encodes a transmembrane domain, resulting in secretion of sCTLA-4 [67]. Notably, studies have revealed that mCTLA-4 and sCTLA-4 are expressed in tumor cells and are associated with the immune escape of tumors [68,69]. Compared with CD28 on the surface of T cells, both mCTLA-4 and sCTLA-4 bind to CD80 and CD86 on antigen-presenting cells (APCs) with higher affinity, thereby preventing T cell activation [70]. Conversely, mCTLA-4 can mediate cell trans-endocytosis to eliminate CD80 and CD86 from the surface of APC, thus blocking the activation of CD28 (+) T cells by these stimulatory receptors [71]. Although the immunosuppressive effect of mCTLA-4 and sCTLA-4 has been confirmed, its association with cancer prognosis remains controversial. To date, it has been revealed that the expression levels of mCTLA-4 and sCTLA-4 on tumors can be positively correlated with survival in esophageal and non-small cell lung cancer [72,73] and negatively correlated with prognosis in nasopharyngeal and colorectal cancer [74,75]. In the present review, we provide evidence in support of this perspective. The number of CD8-positive T cells was significantly reduced in the tumor microenvironment of colorectal cancer tissues presenting CTLA-4 high expression [75], consistent with the immunosuppressive effect of CTLA-4. Therefore, CTLA-4 has been developed as a new target for immunotherapy, and anti-CTLA-4 monoclonal antibodies could effectively induce antibody-dependent cell-mediated cytotoxicity (ADCC) of T cells [76]. However, a clinical trial indicated that patients with melanoma who received anti-CART-4 immunotherapy failed to achieve any significant therapeutic effect [77]. The mechanism of resistance to anti-CART-4 immunotherapy remains unclear. Given that the secreted PD-L1 variant plays a significant role in resistance to anti-PD-L1 immunotherapy and melanoma cells have been shown to express the secreted CART-4, we hypothesized that the secreted CART-4 (sCTLA-4) is involved in resistance to anti-CART-4 immunotherapy. sCTLA-4 and mCTLA-4 have a high degree of homology, both of which can bind to anti-CART-4 antibodies, implying that sCTLA-4 serves as a decoy to combine therapeutic antibodies in the tumor microenvironment, which reduces the availability of therapeutic antibodies to tumor cells expressing mCTLA-4. Moreover, therapeutic antibodies selectively binding sCTLA-4 instead of cell surface mCTLA-4 would be unlikely to induce effective ADCC of T cell target tumors and enhance the ratio of effector T cells to regulatory T cells in the tumor microenvironment [76].

Alternative splicing promotes tumor resistance to chimeric antigen receptor T cell (CAR-T cell) therapy

CAR-T cells are adoptive T cells that express chimeric antigen receptors targeting specific tumor antigens through genetic engineering, commonly used to treat hematological malignancies [78]. CAR-T cells are highly precise and can specifically eliminate tumor cells expressing particular antigens, thereby avoiding the impact on normal cells.

Conversely, the process of CAR-T cell recognition by tumor cell surface antigens does not require the assistance of histocompatibility complex molecules, which effectively avoids immune escape of tumor cells [79]. CD19 antigens on the surface of tumor cells are the most commonly used targets in CAR-T cells.

CART-19 is performed to treat B cell-driven malignancies by expressing a chimeric antigen receptor against CD19 [80]. CART-19 immunotherapy has reported significant clinical effects, which can potentially induce immune effects targeting tumor cells. However, the poor response of some patients to CART-19 and the phenomenon of cancer relapse following CART-19 treatment cannot be ignored. Studies have observed that the loss of epitopes on the tumor cell surface is the primary mechanism of resistance to CART-19 [81]. Increased abundance of CD19 Δex2 and decreased levels of the CD19 full-length transcript were detected in resistant and recurrent samples of patients with B-cell lymphoma. More importantly, it was proven that the FMC63 epitope of CART-19 is encoded by CD19 exon 2, indicating that malignant B cells expressing CD19 Δex2 cannot be recognized by CART-19, resulting in the development of resistance to CART-19. Additional studies have revealed that low SRSF3 expression in malignant B cells induces the production of CD19 Δex2, which was attributed to SRSF3 as a splicing factor involved in the retention of CD19 exon 2 [82]. However, few studies on alternative splicing and adoptive T cell resistance exist, and available reports are mainly restricted to alternative splicing that promotes resistance to CART-19.

Abnormal splicing variants as cancer biomarkers

As described above, several abnormal splicing variants promote malignant progression and development of therapeutic resistance in cancer, which can be employed as potential cancer markers.

In colorectal cancer, the splicing variant Bcl-xL is an oncogenic driver [83]. Interestingly, it was observed that Bcl-xL and MCL-1 L were the most expressed anti-apoptotic mRNA isoforms in a cancer stem cell transcriptome sequencing study [84]. Cancer stem cells are considered a type of cancer-initiating cell and are closely related to malignant progression and drug resistance in cancer. As a typical cancer stem cell surface marker, CD44 has been validated in various types of cancer [85]. CD44 has two types of splicing variants: CD44s and CD44v. CD44v is considered a potential cancer stem cell marker owing to its unique expression pattern and function [86]. Indeed, CD44v, as a cancer stem cell marker, plays a role in colorectal cancer, gastric cancer, and head and neck cancer [87-89]. PD-L1 is an important target for clinical tumor immunotherapy, and its soluble splicing variants are reportedly related to drug resistance. In non-small cell lung cancer patients, high expression of this PD-L1 soluble splicing variant was associated with a poor prognosis [90]. Therefore, malignant progression and drug resistance of the tumor can be predicted by detecting the expression levels of these splice variants in patients with cancer.

Therapeutic strategy of targeting alternative splicing process

Dysregulation of alternative splicing is related to various biological behaviors of tumor cells and promotes resistance to targeted therapy and immunotherapy. It also provides a novel therapeutic strategy for targeting alternative splicing programs for cancer treatment. Alternative splicing events (ASEs) are mediated via spliceosomes and splicing factors, and there two known pathways to block abnormal alternative splicing in cancer. Spliceostatin A (SSA) and E7107 are anti-tumor drugs that block the assembly of spliceosome A. SSA can break the combination of U2 SnRNA with pre-mRNA by altering the conformation of U2 SnRNA and induces U2 SnRNA to be recruited to the incorrect 3′splice site of the intron [91]. Similarly, E7107 disrupts the interaction between U2 SnRNA and BBP by blocking the ATP-dependent conformational change of U2 SnRNA, thereby blocking the assembly of spliceosome A [92]. Isoginkgetin inhibits assembly of spliceosome B by inhibiting recruitment of the U4/U6/U5 tri-snRNP complex [93]. Conversely, isoginkgetin can induce the switch of IL-32 alternative splicing toward IL-32ϒ isoform, and the accumulation of IL-32ϒ leads to caspase-8 mediated apoptosis [94]. However, owing to the cytotoxicity of isoginkgetin, its clinical application remains to be further investigated. Additionally, there exists a promising treatment strategy that inhibits the activity of splicing kinases. Splicing kinases regulate the entire alternative splicing process by modulating SRSF protein phosphorylation. In vitro assays have revealed that gastric cancer cells treated with TG003 or CLK1 siRNA to inhibit the activity of CLK1 show reduced proliferation, invasion, and migration. Thus, CLK1 can be developed as a novel therapeutic target in gastric cancer by employing a personalized tumor explant culture system [95]. Similarly, the inhibition of SRPK1 could simultaneously influence a variety of oncogenic processes, including angiogenesis, apoptosis, proliferation, migration, invasion, and metastasis. Interestingly, targeting SRPK1 could enhance sensitivity to platinum-based chemotherapy in certain carcinomas [96]. Splicing-correcting therapy uses splice-switching oligonucleotides (SSOs) to target splicing defects, which are often used to treat neuromuscular disorders in clinical practice [97]. SSOs are typically designed as single-stranded RNA molecules (15–30 nucleotides long), containing specific sequences that can be used to bind specific splice sites on pre-mRNA, thereby restricting its splicing products toward a specific direction [98]. However, drugs targeting cancer cells with SSOs are still under investigation.

Conclusion and perspectives

A growing body of evidence has demonstrated that dysregulation of ASEs could promote malignant progression and development of tumor therapeutic resistance, while functioning as therapeutic targets for cancer. Abnormal alternative splicing alters the pattern of protein expression in cancer cells, resulting in the production of protein isoforms with abnormal functions and thereby enhancing therapeutic resistance. Targeted therapy and immunotherapy have gradually replaced traditional radiotherapy and chemotherapy, which has become an irresistible trend in tumor treatment. However, abnormal ASEs have become a major obstacle in the clinical application of targeted therapy and immunotherapy by altering the target of drug action, as well the drug signaling pathway. Fortunately, drugs that target alternative splicing are also under development. Among them, SSOs are the most promising therapeutic drugs, with sequences that can be designed to target specific splicing events in cancer cells, thereby inhibiting the production of tumor-friendly protein isoforms and decreasing resistance to targeted therapy and immunotherapy. Thus, a feasible research direction for tumor treatment in the future could employ SSOs with different sequences produced according to tumor heterogeneity. The combined adaptation of this special SSO with immunotherapy drugs and targeted therapy drugs can limit the generation of drug resistance to the greatest extent, while providing individualized and precise treatment plans in patients with cancers to achieve the desired therapeutic effect. Therefore, a major challenge for future research is to define the splicing pattern of different cancers more accurately in order to explore the potential of therapeutically targeting abnormal splicing events.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Kun Deng: Conceptualization, Data curtion, Writing – original draft. **Jingwei Yao:** Writing – review & editing, Software. **Jialu Huang:** Data curtion. **Yubo Ding:** Data curtion. **Jianhong Zuo:** Funding acquisition, Project administration, Writing – review & editing.

References

[1] P. Early, J. Rogers, M. Davis, K. Calame, M. Bond, R. Wall, L. Hood, Two mRNAs can be produced from a single immunoglobulin mu gene by alternative RNA processing pathways, CellCell 20 (2) (1980) 313–319, doi:10.1016/0092-8674(80)90617-0.

[2] A.E.C. Botto, J.C. Muñoz, L.E. Giono, N. Nieto-Moreno, C. Cuenca, A.R. Kornblihtt, M.J. Muñoz, Reciprocal regulation between alternative splicing and the DNA damage response, Genet. Mol. Biol. 43 (1 suppl. 1) (2020) e20190111, doi:10.1590/1678-4685-gmb-2019-0111.

[3] H.K. Kim, M.H.C. Pham, K.S. Ko, B.D. Rhee, J. Han, Alternative splicing isoforms in health and disease, Pflugers Arch.: Eur. J. Physiol. 470 (7) (2018) 995–1016, doi:10.1007/s00424-018-2136-x.

[4] Z. Dlamini, S.C. Tshidino, R. Hull, Abnormalities in alternative splicing of apoptotic genes and cardiovascular diseases, Int. J. Mol. Sci. 16 (11) (2015) 27171–27190, doi:10.3390/ijms161126017.

[5] M. Montes, B.L. Sanford, D.F. Comiskey, D.S. Chandler, RNA splicing and disease: animal models to therapies, Trends Genet.: TIG 35 (1) (2019) 68–87, doi:10.1016/j.tig.2018.10.002.

[6] M. Danan-Gotthold, R. Golan-Gerstl, E. Eisenberg, K. Meir, R. Karni, E.Y. Levanon, Identification of recurrent regulated alternative splicing events across human solid tumors, Nucleic Acids Res. 43 (10) (2015) 5130–5144, doi:10.1093/nar/gkv210.

[7] S. Bonomi, S. Gallo, M. Catillo, D. Pignataro, G. Biamonti, C. Ghigna, Oncogenic alternative splicing switches: role in cancer progression and prospects for therapy, Int. J. Cell Biol. 2013 (2013) 962038, doi:10.1155/2013/962038.

[8] D.P. Corkery, A.C. Holly, S. Lahsaee, G. Dellaire, Connecting the speckles: splicing kinases and their role in tumorigenesis and treatment response, Nucleus (Austin, Tex) 6 (4) (2015) 279–288, doi:10.1080/19491034.2015.1062194.

[9] F. Meyer, Viral interactions with components of the splicing machinery, Prog. Mol. Biol. Transl. Sci. 142 (2016) 241–268, doi:10.1016/bs.pmbts.2016.05.008.

[10] J. Sperling, R. Sperling, Structural studies of the endogenous spliceosome - the supraspliceosome, Methods (San Diego, Calif) 125 (2017) 70–83, doi:10.1016/j.ymeth.2017.04.005.

[11] T.H. Nguyen, W.P. Galej, X.C. Bai, C.G. Savva, A.J. Newman, S.H. Scheres, K. Nagai, The architecture of the spliceosomal U4/U6.U5 tri-snRNP, NatureNature 523 (7558) (2015) 47–52, doi:10.1038/nature14548.

[12] R. Mathew, K. Hartmuth, S. Möhlmann, H. Urlaub, R. Ficner, R. Lührmann, Phosphorylation of human PRP28 by SRPK2 is required for integration of the U4/U6-U5 tri-snRNP into the spliceosome, Nat. Struct. Mol. Biol. 15 (5) (2008) 435–443, doi:10.1038/nsmb.1415.

[13] P. Papasaikas, J. Valcárcel, The spliceosome: the ultimate RNA chaperone and sculptor, Trends Biochem. Sci. 41 (1) (2016) 33–45, doi:10.1016/j.tibs.2015.11.003.

[14] Z. Su, J. Yin, L. Zhao, R. Li, H. Liang, J. Zhang, K. Wang, Lentiviral vector-mediated RBM5 overexpression downregulates EGFR expression in human non-small cell lung cancer cells, World J. Surg. Oncol. 12 (2014) 367, doi:10.1186/1477-7819-12-367.

[15] V.V. Prabhu, N. Devaraj, Regulating RNA binding motif 5 gene expression- a novel therapeutic target for lung cancer, J. Environ. Pathol., Toxicol. Oncol.: Off. Organ Int. Soc. Environ. Toxicol. Cancer 36 (2) (2017) 99–105, doi:10.1615/JEnvironPatholToxicolOncol.2017019366.

[16] L. Zhao, R. Li, C. Shao, P. Li, J. Liu, K. Wang, 3p21.3 tumor suppressor gene RBM5 inhibits growth of human prostate cancer PC-3 cells through apoptosis, World J. Surg. Oncol. 10 (2012) 247, doi:10.1186/1477-7819-10-247.

[17] J.H. Zuo, W. Zhu, M.Y. Li, X.H. Li, H. Yi, G.Q. Zeng, X.X. Wan, Q.Y. He, J.H. Li, J.Q. Qu, Y. Chen, Z.Q. Xiao, Activation of EGFR promotes squamous carcinoma SCC10A cell migration and invasion via inducing EMT-like phenotype change and MMP-9-mediated degradation of E-cadherin, J. Cell. Biochem. 112 (9) (2011) 2508–2517, doi:10.1002/jcb.23175.

[18] A. Maimon, M. Mogilevsky, A. Shilo, R. Golan-Gerstl, A. Obiedat, V. Ben-Hur, I. Lebenthal-Loinger, I. Stein, R. Reich, J. Beenstock, E. Zehorai, C.L. Andersen, K. Thorsen, T.F. Ørntoft, R.J. Davis, B. Davidson, D. Mu, R. Karni, Mnk2 alternative splicing modulates the p38-MAPK pathway and impacts Ras-induced transformation, Cell Rep. 7 (2) (2014) 501–513, doi:10.1016/j.celrep.2014.03.041.

[19] J. Zuo, T. Ishikawa, S. Boutros, Z. Xiao, J.O. Humtsoe, R.H. Kramer, Bcl-2 overexpression induces a partial epithelial to mesenchymal transition and promotes squamous carcinoma cell invasion and metastasis, Mol. Cancer Res. 8 (2) (2010) 170–182, doi:10.1158/1541-7786.Mcr-09-0354.

[20] H. Kędzierska, A. Piekiełko-Witkowska, Splicing factors of SR and hnRNP families as regulators of apoptosis in cancer, Cancer Lett. 396 (2017) 53–65, doi:10.1016/j.canlet.2017.03.013.

[21] A. Tyson-Capper, H. Gautrey, Regulation of Mcl-1 alternative splicing by hnRNP F, H1 and K in breast cancer cells, RNA Biol. 15 (12) (2018) 1448–1457, doi:10.1080/15476286.2018.1551692.

[22] R. Bhattacharya, T. Mitra, S. Ray Chaudhuri, S.S. Roy, Mesenchymal splice isoform of CD44 (CD44s) promotes EMT/invasion and imparts stem-like properties to ovarian cancer cells, J. Cell. Biochem. 119 (4) (2018) 3373–3383, doi:10.1002/jcb.26504.

[23] Q. Chen, M. Gu, Z.K. Cai, H. Zhao, S.C. Sun, C. Liu, M. Zhan, Y.B. Chen, Z. Wang, TGF-β1 promotes epithelial-to-mesenchymal transition and stemness of prostate cancer cells by inducing PCBP1 degradation and alternative splicing of CD44, Cell. Mol. Life Sci.: CMLS (2020), doi:10.1007/s00018-020-03544-5.

[24] X. Ye, Y. Abou-Rayyah, J. Bischoff, A. Ritchie, N.J. Sebire, P. Watts, A.J. Churchill, D.O. Bates, Altered ratios of pro- and anti-angiogenic VEGF-A variants and pericyte expression of DLL4 disrupt vascular maturation in infantile haemangioma, J. Pathol. 239 (2) (2016) 139–151, doi:10.1002/path.4715.

[25] N.N. Mthembu, Z. Mbita, R. Hull, Z. Dlamini, Abnormalities in alternative splicing of angiogenesis-related genes and their role in HIV-related cancers, HIV/AIDS (Auckland, NZ) 9 (2017) 77–93, doi:10.2147/hiv.S124911.

[26] P.M. Biselli-Chicote, J.M. Biselli, B.R. Cunha, R. Castro, J.V. Maniglia, D.S. Neto, E.H. Tajara, J.F. Góis Filho, E.E. Fukuyama, É.C. Pavarino, E.M. Goloni-Bertollo, Overexpression of antiangiogenic vascular endothelial growth factor isoform and splicing regulatory factors in oral, laryngeal and pharyngeal squamous cell carcinomas, Asian Pac. J. Cancer Prev. 18 (8) (2017) 2171–2177, doi:10.22034/apjcp.2017.18.8.2171.

[27] K. Zahra, T. Dey, Ashish, S.P. Mishra, U. Pandey, Pyruvate kinase M2 and cancer: the role of PKM2 in promoting tumorigenesis, Front. Oncol. 10 (2020) 159, doi:10.3389/fonc.2020.00159.

[28] Y. Hu, K. Deng, M. Pan, S. Liu, W. Li, J. Huang, J. Yao, J. Zuo, Down-regulation of PCK2 inhibits the invasion and metastasis of laryngeal carcinoma cells, Am. J. Transl. Res. 12 (7) (2020) 3842–3857.

[29] C. Wang, Y. Li, S. Yan, H. Wang, X. Shao, M. Xiao, B. Yang, G. Qin, R. Kong, R. Chen, N. Zhang, Interactome analysis reveals that lncRNA HULC promotes aerobic glycolysis through LDHA and PKM2, Nat. Commun. 11 (1) (2020) 3162, doi:10.1038/s41467-020-16966-3.

[30] Y. Kuranaga, N. Sugito, H. Shinohara, T. Tsujino, K. Taniguchi, K. Komura, Y. Ito, T. Soga, Y. Akao, SRSF3, a splicer of the PKM gene, regulates cell growth and maintenance of cancer-specific energy metabolism in colon cancer cells, Int. J. Mol. Sci. 19 (10) (2018), doi:10.3390/ijms19103012.

[31] W. Zhu, B.L. Zhou, L.J. Rong, L. Ye, H.J. Xu, Y. Zhou, X.J. Yan, W.D. Liu, B. Zhu, L. Wang, X.J. Jiang, C.P. Ren, Roles of PTBP1 in alternative splicing, glycolysis, and oncogensis, J. Zhejiang Univ. Sci. B 21 (2) (2020) 122–136, doi:10.1631/jzus.B1900422.

[32] Y.T. Lee, Y.J. Tan, C.E. Oon, Molecular targeted therapy: treating cancer with specificity, Eur. J. Pharmacol. 834 (2018) 188–196, doi:10.1016/j.ejphar.2018.07.034.

[33] G.V. Røsland, A.S. Engelsen, Novel points of attack for targeted cancer therapy, Basic Clin. Pharmacol. Toxicol. 116 (1) (2015) 9–18, doi:10.1111/bcpt.12313.

[34] B.D. Wang, N.H. Lee, Aberrant RNA splicing in cancer and drug resistance, Cancers (Basel) 10 (11) (2018), doi:10.3390/cancers10110458.

[35] K. Isobe, A. Kakimoto, T. Mikami, K. Kaburaki, H. Kobayashi, T. Yoshizawa, T. Makino, H. Otsuka, G.O. Sano, K. Sugino, S. Sakamoto, Y. Takai, N. Tochigi, A. Iyoda, S. Homma, Association of BIM deletion polymorphism and BIM-γ RNA expression in NSCLC with EGFR mutation, Cancer Genom. Proteom. 13 (6) (2016) 475–482, doi:10.21873/cgp.20010.

[36] B. Cao, Y. Qi, G. Zhang, D. Xu, Y. Zhan, X. Alvarez, Z. Guo, X. Fu, S.R. Plymate, O. Sartor, H. Zhang, Y. Dong, Androgen receptor splice variants activating the full-length receptor in mediating resistance to androgen-directed therapy, Oncotarget 5 (6) (2014) 1646–1656, doi:10.18632/oncotarget.1802.

[37] Z. Siegfried, R. Karni, The role of alternative splicing in cancer drug resistance, Curr. Opin. Genet. Dev. 48 (2018) 16–21, doi:10.1016/j.gde.2017.10.001.

[38] C. Henry, M. Deschamps, P.S. Rohrlich, J.R. Pallandre, J.P. Rémy-Martin, M. Callanan, A. Traverse-Glehen, C. GrandClément, F. Garnache-Ottou, R. Gressin, E. Deconinck, G. Salles, E. Robinet, P. Tiberghien, C. Borg, C. Ferrand, Identification of an alternative CD20 transcript variant in B-cell malignancies coding for a novel protein associated to rituximab resistance, Blood 115 (12) (2010) 2420–2429, doi:10.1182/blood-2009-06-229112.

[39] C. Gamonet, E. Bole-Richard, A. Delherme, F. Aubin, E. Toussirot, F. Garnache-Ottou, Y. Godet, L. Ysebaert, O. Tournilhac, D. Caroline, F. Larosa, E. Deconinck, P. Saas, C. Borg, M. Deschamps, C. Ferrand, New CD20 alternative splice variants: molecular identification and differential expression within hematological B cell malignancies, Exp. Hematol. Oncol. 5 (2015) 7, doi:10.1186/s40164-016-0036-3.

[40] Y. Zhao, I. Aldoss, C. Qu, J.C. Crawford, Z. Gu, E.K. Allen, A.E. Zamora, T.B. Alexander, J. Wang, H. Goto, T. Imamura, K. Akahane, G. Marcucci, A.S. Stein, R. Bhatia, P. Thomas, S.J. Forman, C.G. Mullighan, K.G. Roberts, Tumor intrinsic and extrinsic determinants of response to blinatumomab in adults with B-ALL, Blood (2020), doi:10.1182/blood.2020006287.

[41] J. Fischer, C. Paret, K. El Malki, F. Alt, A. Wingerter, M.A. Neu, B. Kron, A. Russo, N. Lehmann, L. Roth, E.M. Fehr, S. Attig, A. Hohberger, T. Kindler, J. Faber, CD19 isoforms enabling resistance to CART-19 immunotherapy are expressed in B-ALL patients at initial diagnosis, J. Immunother. (Hagerstown, Md: 1997) 40 (5) (2017) 187–195, doi:10.1097/cji.0000000000000169.

[42] T. Triulzi, G.V. Bianchi, E. Tagliabue, Predictive biomarkers in the treatment of HER2-positive breast cancer: an ongoing challenge, Fut. Oncol. 12 (11) (2016) 1413–1428, doi:10.2217/fon-2015-0025.

[43] D. Mitra, M.J. Brumlik, S.U. Okamgba, Y. Zhu, T.T. Duplessis, J.G. Parvani, S.M. Lesko, E. Brogi, F.E. Jones, An oncogenic isoform of HER2 associated with locally disseminated breast cancer and trastuzumab resistance, Mol. Cancer Ther. 8 (8) (2009) 2152–2162, doi:10.1158/1535-7163.Mct-09-0295.

[44] J. Turpin, C. Ling, E.J. Crosby, Z.C. Hartman, A.M. Simond, L.A. Chodosh, J.P. Rennhack, E.R. Andrechek, J. Ozcelik, M. Hallett, G.B. Mills, R.D. Cardiff, J.W. Gray, O.L. Griffith, W.J. Muller, The ErbB2ΔEx16 splice variant is a major oncogenic driver in breast cancer that promotes a pro-metastatic tumor microenvironment, Oncogene 35 (47) (2016) 6053–6064, doi:10.1038/onc.2016.129.

[45] A. Alajati, N. Sausgruber, N. Aceto, S. Duss, S. Sarret, H. Voshol, D. Bonenfant, M. Bentires-Alj, Mammary tumor formation and metastasis evoked by a HER2 splice variant, Cancer Res. 73 (17) (2013) 5320–5327, doi:10.1158/0008-5472.Can-12-3186.

[46] S. Goel, D.G. Duda, L. Xu, L.L. Munn, Y. Boucher, D. Fukumura, R.K. Jain, Normalization of the vasculature for treatment of cancer and other diseases, Physiol. Rev. 91 (3) (2011) 1071–1121, doi:10.1152/physrev.00038.2010.

[47] S. Pennacchietti, P. Michieli, M. Galluzzo, M. Mazzone, S. Giordano, P.M. Comoglio, Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene, Cancer Cell 3 (4) (2003) 347–361, doi:10.1016/s1535-6108(03)00085-0.

[48] J. Zuo, J. Wen, M. Lei, M. Wen, S. Li, X. Lv, Z. Luo, G. Wen, Hypoxia promotes the invasion and metastasis of laryngeal cancer cells via EMT, Med. Oncol. 33 (2) (2016) 15, doi:10.1007/s12032-015-0716-6.

[49] R.T. Tong, Y. Boucher, S.V. Kozin, F. Winkler, D.J. Hicklin, R.K. Jain, Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors, Cancer Res. 64 (11) (2004) 3731–3736, doi:10.1158/0008-5472.Can-04-0074.

[50] A. Palladini, G. Nicoletti, A. Lamolinara, M. Dall'Ora, T. Balboni, M.L. Ianzano, R. Laranga, L. Landuzzi, V. Giusti, C. Ceccarelli, D. Santini, M. Taffurelli, E. Di Oto, S. Asioli, A. Amici, S.M. Pupa, C. De Giovanni, E. Tagliabue, M. Iezzi, P. Nanni, P.L. Lollini, HER2 isoforms co-expression differently tunes mammary tumor phenotypes affecting onset, vasculature and therapeutic response, Oncotarget 8 (33) (2017) 54444–54458, doi:10.18632/oncotarget.17088.

[51] P.B. Chapman, Mechanisms of resistance to RAF inhibition in melanomas harboring a BRAF mutation, American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting, 2013, doi:10.1200/EdBook_AM.2013.33.e80.

[52] P.I. Poulikakos, Y. Persaud, M. Janakiraman, X. Kong, C. Ng, G. Moriceau, H. Shi, M. Atefi, B. Titz, M.T. Gabay, M. Salton, K.B. Dahlman, M. Tadi, J.A. Wargo, K.T. Flaherty, M.C. Kelley, T. Misteli, P.B. Chapman, J.A. Sosman, T.G. Graeber, A. Ribas, R.S. Lo, N. Rosen, D.B. Solit, RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E), NatureNature 480 (7377) (2011) 387–390, doi:10.1038/nature10662.

[53] M. Panda, B.K. Biswal, Cell signaling and cancer: a mechanistic insight into drug resistance, Mol. Biol. Rep. 46 (5) (2019) 5645–5659, doi:10.1007/s11033-019-04958-6.

[54] Z.Y. Wang, L. Yin, Estrogen receptor alpha-36 (ER-α36): a new player in human breast cancer, Mol. Cell. Endocrinol. 418 (Pt 3) (2015) 193–206, doi:10.1016/j.mce.2015.04.017.

[55] K. Inoue, E.A. Fry, Aberrant splicing of estrogen receptor, HER2, and CD44 genes in breast cancer, Genet. Epigenet. 7 (2015) 19–32, doi:10.4137/geg.S35500.

[56] L. Yin, Z.Y. Wang, Roles of the ER-α36-EGFR/HER2 positive regulatory loops in tamoxifen resistance, Steroids 111 (2016) 95–99, doi:10.1016/j.steroids.2016.01.019.

[57] M.A. Maczis, M. Maceyka, M.R. Waters, J. Newton, M. Singh, M.F. Rigsby, T.H. Turner, M.A. Alzubi, J.C. Harrell, S. Milstien, S. Spiegel, Sphingosine kinase 1 activation by estrogen receptor α36 contributes to tamoxifen resistance in breast cancer, J. Lipid Res. 59 (12) (2018) 2297–2307, doi:10.1194/jlr.M085191.

[58] P. Sharma, S. Hu-Lieskovan, J.A. Wargo, A. Ribas, Primary, adaptive, and acquired resistance to cancer immunotherapy, CellCell 168 (4) (2017) 707–723, doi:10.1016/j.cell.2017.01.017.

[59] R. Bai, N. Chen, L. Li, N. Du, L. Bai, Z. Lv, H. Tian, J. Cui, Mechanisms of cancer resistance to immunotherapy, Front. Oncol. 10 (2020) 1290, doi:10.3389/fonc.2020.01290.

[60] Y. Wang, H. Zhang, B. Jiao, J. Nie, X. Li, W. Wang, H. Wang, The roles of alternative splicing in tumor-immune cell interactions, Curr. Cancer Drug Targets (2020), doi:10.2174/1568009620666200619123725.

[61] T. Yokosuka, M. Takamatsu, W. Kobayashi-Imanishi, A. Hashimoto-Tane, M. Azuma, T. Saito, Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2, J. Exp. Med. 209 (6) (2012) 1201–1217, doi:10.1084/jem.20112741.

[62] N.B. Hassounah, V.S. Malladi, Y. Huang, S.S. Freeman, E.M. Beauchamp, S. Koyama, N. Souders, S. Martin, G. Dranoff, K.K. Wong, C.S. Pedamallu, P.S. Hammerman, E.A. Akbay, Identification and characterization of an alternative cancer-derived PD-L1 splice variant, Cancer Immunol. Immunother. 68 (3) (2019) 407–420, doi:10.1007/s00262-018-2284-z.

[63] B. Gong, K. Kiyotani, S. Sakata, S. Nagano, S. Kumehara, S. Baba, B. Besse, N. Yanagitani, L. Friboulet, M. Nishio, K. Takeuchi, H. Kawamoto, N. Fujita, R. Katayama, Secreted PD-L1 variants mediate resistance to PD-L1 blockade therapy in non-small cell lung cancer, J. Exp. Med. 216 (4) (2019) 982–1000, doi:10.1084/jem.20180870.

[64] C. Nielsen, L. Ohm-Laursen, T. Barington, S. Husby, S.T. Lillevang, Alternative splice variants of the human PD-1 gene, Cell. Immunol. 235 (2) (2005) 109–116, doi:10.1016/j.cellimm.2005.07.007.

[65] J. Sun, J. Bai, T. Jiang, Y. Gao, Y. Hua, Modulation of PDCD1 exon 3 splicing, RNA Biol. 16 (12) (2019) 1794–1805, doi:10.1080/15476286.2019.1659080.

[66] E.A. Tivol, J. Gorski, *Re*-establishing peripheral tolerance in the absence of CTLA-4: complementation by wild-type T cells points to an indirect role for CTLA-4, J. Immunol. 169 (4) (2002) 1852–1858, doi:10.4049/jimmunol.169.4.1852.

[67] M.K. Oaks, K.M. Hallett, R.T. Penwell, E.C. Stauber, S.J. Warren, A.J. Tector, A native soluble form of CTLA-4, Cell. Immunol. 201 (2) (2000) 144–153, doi:10.1006/cimm.2000.1649.

[68] E. Contardi, G.L. Palmisano, P.L. Tazzari, A.M. Martelli, F. Falà, M. Fabbi, T. Kato, E. Lucarelli, D. Donati, L. Polito, A. Bolognesi, F. Ricci, S. Salvi, V. Gargaglione, S. Mantero, M. Alberghini, G.B. Ferrara, M.P. Pistillo, CTLA-4 is constitutively expressed on tumor cells and can trigger apoptosis upon ligand interaction, Int. J. Cancer 117 (4) (2005) 538–550, doi:10.1002/ijc.21155.

[69] S. Laurent, P. Queirolo, S. Boero, S. Salvi, P. Piccioli, S. Boccardo, S. Minghelli, A. Morabito, V. Fontana, G. Pietra, P. Carrega, N. Ferrari, F. Tosetti, L.J. Chang, M.C. Mingari, G. Ferlazzo, A. Poggi, M.P. Pistillo, The engagement of CTLA-4 on primary melanoma cell lines induces antibody-dependent cellular cytotoxicity and TNF-α production, J. Transl. Med. 11 (2013) 108, doi:10.1186/1479-5876-11-108.

[70] C.E. Rudd, A. Taylor, H. Schneider, CD28 and CTLA-4 coreceptor expression and signal transduction, Immunol. Rev. 229 (1) (2009) 12–26, doi:10.1111/j.1600-065X.2009.00770.x.

[71] O.S. Qureshi, Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E.M. Schmidt, J. Baker, L.E. Jeffery, S. Kaur, Z. Briggs, T.Z. Hou, C.E. Futter, G. Anderson, L.S. Walker, D.M. Sansom, Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4, Science 332 (6029) (2011) 600–603, doi:10.1126/science.1202947.

[72] Q. Liu, P. Hu, G. Deng, J. Zhang, N. Liang, J. Xie, L. Qiao, H. Luo, J. Zhang, Soluble cytotoxic T-lymphocyte antigen 4: a favorable predictor in malignant tumors after therapy, Onco Targets Ther. 10 (2017) 2147–2154, doi:10.2147/ott.S128451.

[73] S. Salvi, V. Fontana, S. Boccardo, D.F. Merlo, E. Margallo, S. Laurent, A. Morabito, E. Rijavec, M.G. Dal Bello, M. Mora, G.B. Ratto, F. Grossi, M. Truini, M.P. Pistillo, Evaluation of CTLA-4 expression and relevance as a novel prognostic factor in patients with non-small cell lung cancer, Cancer Immunol. Immunother. 61 (9) (2012) 1463–1472, doi:10.1007/s00262-012-1211-y.

[74] P.Y. Huang, S.S. Guo, Y. Zhang, J.B. Lu, Q.Y. Chen, L.Q. Tang, L. Zhang, L.T. Liu, L. Zhang, H.Q. Mai, Tumor CTLA-4 overexpression predicts poor survival in patients with nasopharyngeal carcinoma, Oncotarget 7 (11) (2016) 13060–13068, doi:10.18632/oncotarget.7421.

[75] Y. Omura, Y. Toiyama, Y. Okugawa, C. Yin, T. Shigemori, K. Kusunoki, Y. Kusunoki, S. Ide, T. Shimura, H. Fujikawa, H. Yasuda, J. Hiro, M. Ohi, M. Kusunoki, Prognostic impacts of tumoral expression and serum levels of PD-L1 and CTLA-4 in colorectal cancer patients, Cancer Immunol. Immunother. (2020), doi:10.1007/s00262-020-02645-1.

[76] T.R. Simpson, F. Li, W. Montalvo-Ortiz, M.A. Sepulveda, K. Bergerhoff, F. Arce, C. Roddie, J.Y. Henry, H. Yagita, J.D. Wolchok, K.S. Peggs, J.V. Ravetch, J.P. Allison, S.A. Quezada, Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma, J. Exp. Med. 210 (9) (2013) 1695–1710, doi:10.1084/jem.20130579.

[77] A. Ribas, R. Kefford, M.A. Marshall, C.J. Punt, J.B. Haanen, M. Marmol, C. Garbe, H. Gogas, J. Schachter, G. Linette, P. Lorigan, K.L. Kendra, M. Maio, U. Trefzer, M. Smylie, G.A. McArthur, B. Dreno, P.D. Nathan, J. Mackiewicz, J.M. Kirkwood, J. Gomez-Navarro, B. Huang, D. Pavlov, A. Hauschild, Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma, J. Clin. Oncol. 31 (5) (2013) 616–622, doi:10.1200/jco.2012.44.6112.

[78] R. Mohanty, C.R. Chowdhury, S. Arega, P. Sen, P. Ganguly, N. Ganguly, CAR T cell therapy: a new era for cancer treatment (Review), Oncol. Rep. 42 (6) (2019) 2183–2195, doi:10.3892/or.2019.7335.

[79] Z. Zhao, Y. Chen, N.M. Francisco, Y. Zhang, M. Wu, The application of CAR-T cell therapy in hematological malignancies: advantages and challenges, Acta Pharm. Sin. B 8 (4) (2018) 539–551, doi:10.1016/j.apsb.2018.03.001.

[80] S. Jasinski, F.A. De Los Reyes, G.C. Yametti, J. Pierro, E. Raetz, W.L. Carroll, Immunotherapy in pediatric B-cell acute lymphoblastic leukemia: advances and ongoing challenges, Paediatr. Drugs 22 (5) (2020) 485–499, doi:10.1007/s40272-020-00413-3.

[81] N.N. Shah, T. Maatman, P. Hari, B. Johnson, Multi targeted CAR-T cell therapies for B-cell malignancies, Front. Oncol. 9 (2019) 146, doi:10.3389/fonc.2019.00146.

[82] E. Sotillo, D.M. Barrett, K.L. Black, A. Bagashev, D. Oldridge, G. Wu, R. Sussman, C. Lanauze, M. Ruella, M.R. Gazzara, N.M. Martinez, C.T. Harrington, E.Y. Chung, J. Perazzelli, T.J. Hofmann, S.L. Maude, P. Raman, A. Barrera, S. Gill, S.F. Lacey, J.J. Melenhorst, D. Allman, E. Jacoby, T. Fry, C. Mackall, Y. Barash, K.W. Lynch, J.M. Maris, S.A. Grupp, A. Thomas-Tikhonenko, Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy, Cancer Discov. 5 (12) (2015) 1282–1295, doi:10.1158/2159-8290.Cd-15-1020.

[83] A.L. Scherr, G. Gdynia, M. Salou, P. Radhakrishnan, K. Duglova, A. Heller, S. Keim, N. Kautz, A. Jassowicz, C. Elssner, Y.W. He, D. Jaeger, M. Heikenwalder, M. Schneider, A. Weber, W. Roth, H. Schulze-Bergkamen, B.C. Koehler, Bcl-xL is an oncogenic driver in colorectal cancer, Cell Death Dis. 7 (8) (2016) e2342, doi:10.1038/cddis.2016.233.

[84] C.L. Barrett, R.B. Schwab, H. Jung, B. Crain, D.J. Goff, C.H. Jamieson, P.A. Thistlethwaite, O. Harismendy, D.A. Carson, K.A. Frazer, Transcriptome sequencing of tumor subpopulations reveals a spectrum of therapeutic options for squamous cell lung cancer, PLoS ONE 8 (3) (2013) e58714, doi:10.1371/journal.pone.0058714.

[85] Y. Yan, X. Zuo, D. Wei, Concise review: emerging role of CD44 in cancer stem cells: a promising biomarker and therapeutic target, Stem Cells Transl. Med. 4 (9) (2015) 1033–1043, doi:10.5966/sctm.2015-0048.

[86] L. Wang, X. Zuo, K. Xie, D. Wei, The role of CD44 and cancer stem cells, Methods Mol. Biol. 1692 (2018) 31–42, doi:10.1007/978-1-4939-7401-6_3.

[87] M. Todaro, M. Gaggianesi, V. Catalano, A. Benfante, F. Iovino, M. Biffoni, T. Apuzzo, I. Sperduti, S. Volpe, G. Cocorullo, G. Gulotta, F. Dieli, R. De Maria, G. Stassi, CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis, Cell Stem Cell 14 (3) (2014) 342–356, doi:10.1016/j.stem.2014.01.009.

[88] W.M. Lau, E. Teng, H.S. Chong, K.A. Lopez, A.Y. Tay, M. Salto-Tellez, A. Shabbir,

J.B. So, S.L. Chan, CD44v8-10 is a cancer-specific marker for gastric cancer stem cells, Cancer Res. 74 (9) (2014) 2630–2641, doi:10.1158/0008-5472.Can-13-2309.
[89] L.Y. Bourguignon, G. Wong, C. Earle, L. Chen, Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma, J. Biol. Chem. 287 (39) (2012) 32800–32824, doi:10.1074/jbc.M111.308528.
[90] J. Zhang, J. Gao, Y. Li, J. Nie, L. Dai, W. Hu, X. Chen, J. Han, X. Ma, G. Tian, D. Wu, L. Shen, J. Fang, Circulating PD-L1 in NSCLC patients and the correlation between the level of PD-L1 expression and the clinical characteristics, Thorac Cancer 6 (4) (2015) 534–538, doi:10.1111/1759-7714.12247.
[91] M. Koga, T. Satoh, I. Takasaki, Y. Kawamura, M. Yoshida, D. Kaida, U2 snRNP is required for expression of the 3′ end of genes, PLoS ONE 9 (5) (2014) e98015, doi:10.1371/journal.pone.0098015.
[92] E.G. Folco, K.E. Coil, R. Reed, The anti-tumor drug E7107 reveals an essential role for SF3b in remodeling U2 snRNP to expose the branch point-binding region, Genes Dev. 25 (5) (2011) 440–444, doi:10.1101/gad.2009411.
[93] E.J. Vanzyl, K.R.C. Rick, A.B. Blackmore, E.M. MacFarlane, B.C. McKay, Flow cytometric analysis identifies changes in S and M phases as novel cell cycle alterations induced by the splicing inhibitor isoginkgetin, PLoS ONE 13 (1) (2018) e0191178, doi:10.1371/journal.pone.0191178.
[94] B. Heinhuis, T.S. Plantinga, G. Semango, B. Küsters, M.G. Netea, C.A. Dinarello, J.W.A. Smit, R.T. Netea-Maier, L.A.B. Joosten, Alternatively spliced isoforms of IL-32 differentially influence cell death pathways in cancer cell lines, Carcinogenesis 37 (2) (2016) 197–205, doi:10.1093/carcin/bgv172.
[95] N. Babu, S.M. Pinto, M. Biswas, T. Subbannayya, M. Rajappa, S.V. Mohan, J. Advani, P. Rajagopalan, G. Sathe, N. Syed, V.D. Radhakrishna, O. Muthusamy, S. Navani, R.V. Kumar, G. Gopisetty, T. Rajkumar, P. Radhakrishnan, S. Thiyagarajan, A. Pandey, H. Gowda, P. Majumder, A. Chatterjee, Phosphoproteomic analysis identifies CLK1 as a novel therapeutic target in gastric cancer, Gastr. Cancer: Off. J. Int. Gastr. Cancer Assoc. Japan. Gastr. Cancer Assoc. (2020), doi:10.1007/s10120-020-01062-8.
[96] I.P. Nikas, S.C. Themistocleous, S.A. Paschou, K.I. Tsamis, H.S. Ryu, Serine-arginine protein kinase 1 (SRPK1) as a prognostic factor and potential therapeutic target in cancer: current evidence and future perspectives, Cells 9 (1) (2019), doi:10.3390/cells9010019.
[97] F. Bizot, A. Vulin, A. Goyenvalle, Current status of antisense oligonucleotide-based therapy in neuromuscular disorders, Drugs (2020), doi:10.1007/s40265-020-01363-3.
[98] M.A. Havens, M.L. Hastings, Splice-switching antisense oligonucleotides as therapeutic drugs, Nucleic Acids Res. 44 (14) (2016) 6549–6563, doi:10.1093/nar/gkw533.