Oncogene (2015) **34,** 1–14

www.nature.com/onc

REVIEW

Alternative splicing in cancer: implications for biology and therapy

J Chen[1,2] and WA Weiss[2,3]

Alternative splicing has critical roles in normal development and can promote growth and survival in cancer. Aberrant splicing, the production of noncanonical and cancer-specific mRNA transcripts, can lead to loss-of-function in tumor suppressors or activation of oncogenes and cancer pathways. Emerging data suggest that aberrant splicing products and loss of canonically spliced variants correlate with stage and progression in malignancy. Here, we review the splicing landscape of *TP53*, *BARD1* and *AR* to illuminate roles for alternative splicing in cancer. We also examine the intersection between alternative splicing pathways and novel therapeutic approaches.

Oncogene (2015) **34,** 1–14; doi:10.1038/onc.2013.570; published online 20 January 2014

Keywords: alternative splicing; aberrant splicing; p53; BARD1; AR

INTRODUCTION

Alternative splicing generates multiple different mRNAs and downstream proteins from a single gene through the inclusion or exclusion of specific exons. This process occurs in 95% of all multi-exonic genes[1] and is catalyzed by the spliceosome, a complex comprised from a core of five small nuclear ribonucleoproteins (U1, U2, U4, U5 and U6; reviewed in Burge *et al.*[2]). The spliceosome is aided by over 200 *trans*-acting factors that recognize *cis*-regulatory sequences within the pre-mRNA and direct the spliceosome to include or exclude specific exons (reviewed in Chen and Manley[3] and Wahl *et al.*[4]). Splice variants can thus arise from mechanisms including alternative promoters, preferential usage of exons or splice sites, scrambling of exon order and alternative polyadenylation (Figure 1).

Alternative splicing affords a significant evolutionary advantage by providing a large source of proteomic diversity.[5] Alternative splicing is often regulated at the tissue level, and tissue-specific variants cooperate to modulate protein–protein interaction networks.[6] Stem cells express specific splice variants at each stage of differentiation, with undifferentiated stem cells maintaining the highest levels of diversity for splice isoforms.[7] Alternative splicing is also critical in development and can be responsive to normal external stimuli (reviewed in Kalsotra and Cooper[8] and Heyd and Lynch[9]). As with other developmentally related pathways, alternative splicing can be aberrantly regulated by cancer cells to their advantage. Genome-wide studies have long revealed the existence of cancer-specific splicing patterns.[10–12] The ability to commandeer alternative splicing could be beneficial to cancer cells if early developmental stage isoforms critical for proliferation are also ectopically expressed, driving uncontrolled growth. This switch in splicing preference can be critical as numerous genes possess splice variants that are mutually antagonistic.

Escape from cell death is critical for tumorigenesis and some alternatively spliced genes in cancer modulate apoptosis. For example, *BCL2L1* possesses an alternative 5′ splice site after exon 2 that produces long and short isoforms that are translated into the BCL-X_L and BCL-X_S proteins, respectively (Figure 2a). BCL-X_S promotes apoptosis whereas BCL-X_L has anti-apoptotic effects.[13] Cancers show predominant expression of the BCL-X_L isoform.[14,15] Similarly, the FAS receptor (*TNR6*) is a cell surface receptor that can initiate cell death when bound to TNFS6, the FAS ligand.[16] *TNR6* is subject to alternative splicing (Figure 2b); in particular, a splice variant lacking the transmembrane domain results from exon skipping at exon 6.[17] This shorter product is soluble and inhibits FAS-mediated cell death, presumably by binding competition for FAS ligand.[18] Soluble FAS is detectable in serum, with higher concentrations found in cancer patients compared with healthy individuals.[19–21]

Metabolic pathways are also frequently altered in cancer. The Warburg effect, a shift from oxidative phosphorylation to aerobic glycolysis, enables synthetic pathways at the expense of ATP production in cancer cells[22] and is partly driven by alternative splicing of the pyruvate kinase M gene (*PKM*). Exons 9 and 10 are mutually exclusive (Figure 2c), giving rise to *PKM1* (exon 9), the adult isoform, or *PKM2* (exon 10), the embryonic or tumor isoform.[23] PKM2 is expressed widely in cancer (reviewed in Mazurek *et al.*[24]), and replacement of PKM2 with PKM1 reverses the Warburg effect and increases oxidative phosphorylation.[25]

The regulation between antagonistic splice variants of the same gene can also be disrupted to affect proliferative pathways, interactions with proto-oncogenes and tumor suppressors, and the epithelial to mesenchymal transition, a pathway that promotes invasion and metastasis. Although the switch between antagonistic gene isoforms in cancer illustrates critical roles for alternative splicing, this subject has been covered recently[26] and will not be reviewed here.

These splice variants are considered canonical products of alternative splicing because they are expressed in somatic tissue as part of normal development or to facilitate cell-specific functions. Although the definition of canonical splicing patterns

[1]Biomedical Sciences Graduate Program, University of California, San Francisco, CA, USA; [2]Department of Neurology, University of California, San Francisco, CA, USA and [3]Department of Neurological Surgery and Pediatrics, University of California, San Francisco, CA, USA. Correspondence: J Chen, Department of Neurology, University of California, San Francisco, Box 0520, 1450 3rd Street HD-220, San Francisco, CA 94158, USA.
E-mail: justin.chen@ucsf.edu
Received 17 October 2013; revised 26 November 2013; accepted 26 November 2015; published online 20 January 2014

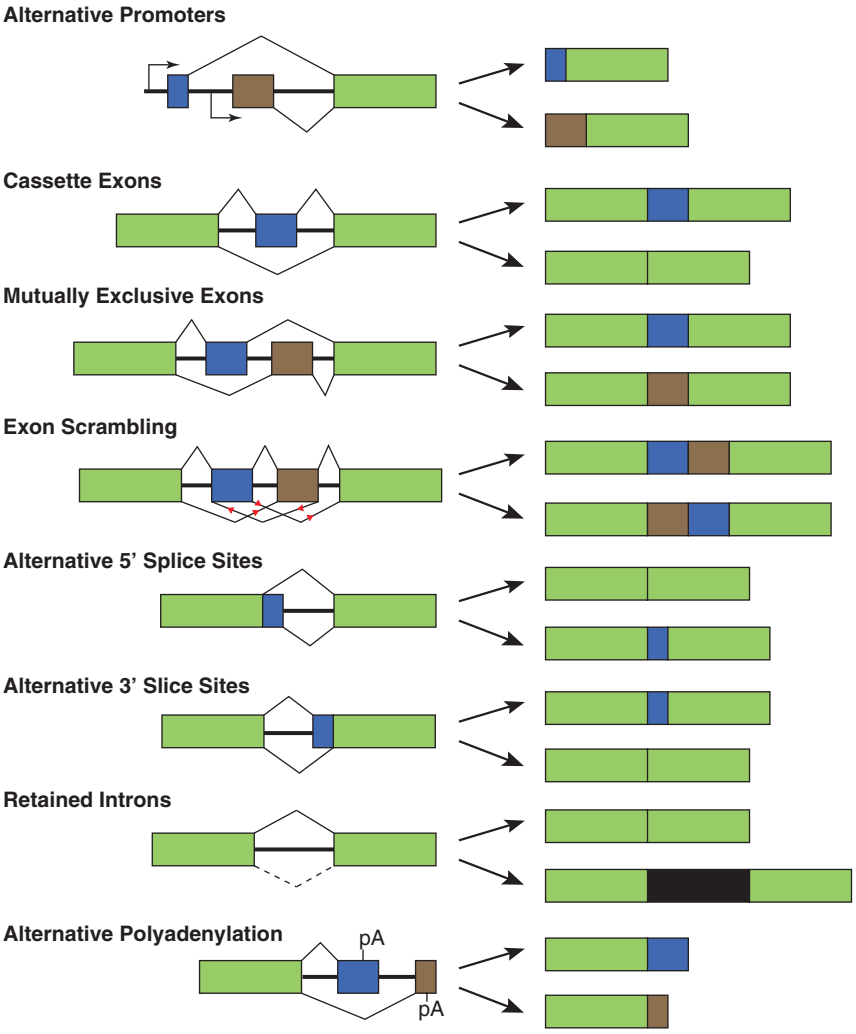

Figure 1. Common mechanisms of alternative splicing. Alternative splicing can occur although a number of different processes and give rise to different mature transcripts (right). Exons and final transcripts are illustrated as boxes while lines represent introns. Constitutively expressed exons are depicted in green, and alternatively spliced exons are depicted in blue or brown. Retained introns occur with the absence of splicing, with the intervening intron (black) included in the final transcript.

will continue to evolve as transcript isoforms are dissected in more detail, alternative splicing does not need to follow these patterns to provide a tumorigenic advantage. This process may be harnessed to produce novel exon combinations and transcripts that are rarely, if ever seen in normal somatic cells. We focus here on examples of this *aberrant* splicing and its association with outcome as well as the therapeutic impact of the alternative splicing pathway. Aberrant splicing can arise through a couple of mechanistic models: alterations in core spliceosomal components can lead to global splicing deregulation and result in a large number of aberrant products. Similarly, alterations in an accessory splicing factor can lead to deregulation of splicing for the limited set of transcripts where the factor is required for accurate splicing. Alternatively, genomic mutations in a critical splicing motif of a single gene will change the splicing pattern of just that transcript.

Regardless of the mechanism, aberrant splicing of proto-oncogenes can produce constitutively active or even gain-of-function variants that confer new survival or proliferative abilities. In contrast, a tumor suppressor with aberrant splicing could result in an altered reading frame and a premature stop codon; subsequent destruction by the nonsense-mediated decay process would ultimately lower the total protein level and its tumor-suppressive capacity. Still, some transcripts may manage to avoid nonsense-mediated decay, and if the truncated protein gains dominant-negative regulatory functions, tumor suppression would be lowered even further. Until recently, technical reasons have led researchers to focus primarily on nonsynonymous coding mutations with easily predictable consequences. By doing so, the single largest source of protein diversity has been woefully neglected. This is best exemplified by the fact that there is perhaps no tumor suppressor as prominent as p53, yet its splicing has only recently gained significant appreciation.

P53

P53 is encoded by the gene *TP53* and is one of the most well-known and well-studied tumor suppressors since its discovery over 30 years ago.[27,28] Although it was originally considered an oncogene for nearly a decade,[29–31] p53 is a transcription factor[32,33] that enacts tumor suppression by mediating cellular functions including, but not limited to, apoptosis,[34,35] cellular senescence[36,37] and DNA repair[38] (reviewed in Levine and Oren[31] and Vogelstein *et al.*[39]). P53 activity is largely governed through a negative feedback loop with MDM2,[40–42] an E3 ubiquitin-protein

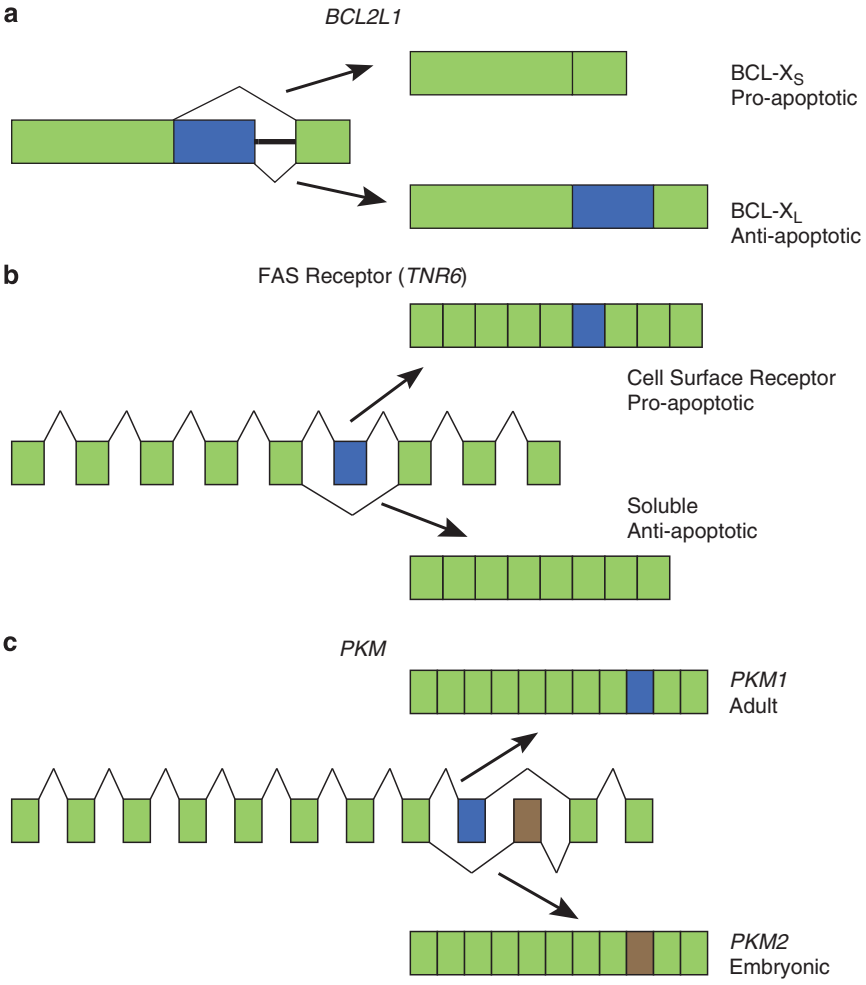

Figure 2. Alternative splicing leads to antagonistic variants. (**a**) *BCL2L1* is spliced into two variants based on an alternative 5′ splice site (blue). Expression of the two canonical exons (green) results in the pro-apoptotic BCL-X_S short isoform, whereas the long isoform, BCL-X_L, arises from the splice site downstream of exon 1 and is anti-apoptotic. Exon sizes are relative to actual length, but the intron is not drawn to scale. (**b**) Exon 6 (blue) of *TNR6* is a cassette exon. Inclusion in the mature mRNA leads to expression of the FAS receptor on the cell surface where it mediates cell death. Exclusion of exon 6 results in a soluble FAS isoform, which has anti-apoptotic effects by competing for ligand. Exons and introns are not drawn to scale. (**c**) *PKM* exons 9 (blue) and 10 (brown) are mutually exclusive exons. The embryonic variant *PKM2* arises with incorporation of exon 10. Upon maturation to adulthood, this variant is typically switched to *PKM1* by the alternative incorporation of exon 9. *PKM2* promotes aerobic glycolysis whereas *PKM1* promotes oxidative phosphorylation. Exons and introns are not drawn to scale.

ligase that mediates proteasomal degradation of p53. Mutations in p53 that disrupt either of the tandem transactivating domains often contribute to tumorigeneisis (reviewed in Brosh and Rotter[43]).

Alternative splicing of *TP53* was originally identified over 20 years ago in both mouse and humans,[44,45] however, investigation into the functional roles of these splice variants is still ongoing. *TP53* contains 11 exons, which encode major functional domains including: the two tandem transactivation domains (TAD), the first of which also serves as the binding domain for MDM2, the DNA-binding domain and the oligomerization domain (Figure 3a). Owing to intron inclusion, normal human lymphocytes express an alternatively spliced variant of *TP53* with a truncated C-terminal domain. This isoform is found in significant amounts only in quiescent cells and provided the first observation that *TP53* splicing could have functional consequences; presumably due to the truncation of the oligomerization domain,[46] it fails to bind DNA and possesses a transcriptional defect. Subsequently, an N-terminal truncated isoform, Δ40-p53, was discovered in the breast cancer cell line 21PT, arising from an internal transcriptional start site in the first exon.[47] Additional splice variants carrying N-terminal deletions arise from an internal promoter before exon 5 to produce Δ133-p53 and Δ160-p53 isoforms that utilize start methionines at amino-acid positions 133 and 160, respectively[48] (Figure 3b). Interestingly, local internal ribosomal entry sequences have been shown to produce N-terminal truncated isoforms, indicating the need for further study into how these isoforms are generated.[49,50] All three of these N-terminal variants can theoretically combine with three different C-terminal variants that arise from alternative splicing downstream of exon 9 (α, β and γ).[48,51] Canonical splicing of the transcript after exon 9 leads to the α-isoform with a complete oligomerization domain and the inclusion of exons 10 and 11. The β- and γ-isoforms are formed because of intron retention and stop codons, which follow after translation of 10 or 15 new amino acids, respectively (Figure 3c). Coexpression of full-length p53 and p53β enhances transcriptional activity on the *p21* promoter but not the *BAX* promoter, and the two isoforms cooperate to promote senescence.[52]

Additional C-terminal variants were described recently. P53δ and p53ζ arise because of splice site point mutations and result in intron retention. P53δ, similar to the β- and γ-isoforms, truncates the oligomerization domain after exons 9 and 27 new amino acids.

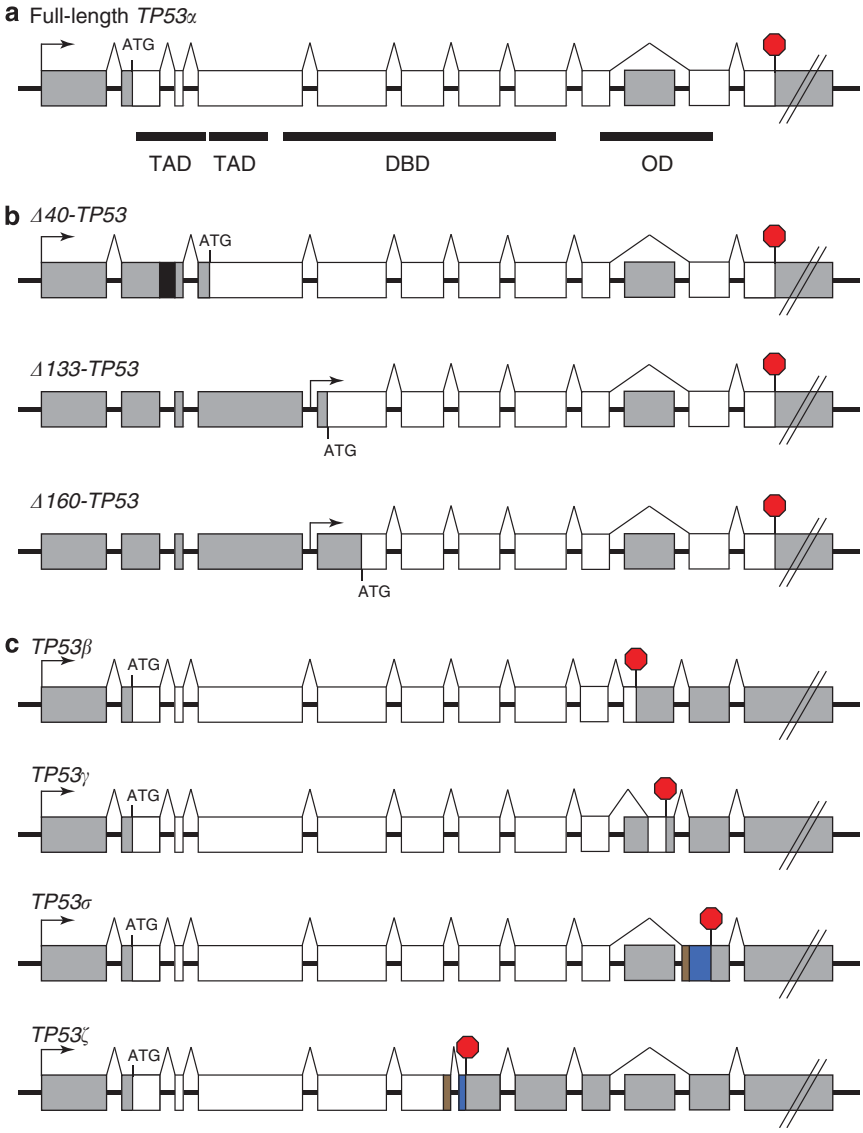

Figure 3. Splicing schematic of *TP53*. (**a**) Exon structure of full-length *TP53* with relative locations encoding the transactivation domains (TAD), DNA-binding domain (DBD) and oligomerization domain (OD) indicated (as described in UniProt). Exon sizes are relative to actual length, but introns are not drawn to scale. Start and stop codons are located at the indicated positions. Coding sequences are indicated as white-filled boxes, and untranslated regions are indicated as gray-filled boxes. Splice variants containing combinations between **b** and **c** have also been identified. (**b**) N-terminal *TP53* splice variants. An alternative promoter between exons 4 and 5 leads to variants that utilize different start codons. Intron retention is indicated by the black-filled box. (**c**) C-terminal *TP53* splice variants. Alternative 5′ or 3′ splice sites are indicated by brown-filled boxes, and alternative reading frames are indicated by blue-filled boxes.

Intron retention in p53ζ results in a frame-shift and a premature termination codon in the middle of exon 7.[53]

In omitting the first 39–159 amino acids and truncating the transactivating domain, N-terminal variants of p53 likely act as dominant-negative regulators. Δ133-p53 inhibits full-length p53 activity as shown by *in vitro* reporter assays.[48,54] Additional *in vitro* studies indicate that Δ133-p53α does not bind p53 response elements and inhibits full-length p53 from binding p53 response elements.[55] Δ133-p53 is expressed in a p53-dependent manner,[56] raising the possibility that this isoform participates in a negative feedback loop to modulate full-length p53 activity. However, by encoding for an earlier translational start site, Δ40-p53 still retains the second TAD, and in a study analyzing p53 mutations within the TADs, activity of just the second TAD allowed for minimal transactivation of most p53 target genes.[57] Indeed, the Δ40-p53 has impaired transcriptional activation and impairs growth suppression by oligomerizing with full-length p53.[47,58] In p53-null cell lines, transfection of Δ40-p53 alone was insufficient to initiate transcriptional activity of a p53 response element reporter, whereas co-transfection with full-length p53 decreased total p53 transcriptional activity in a dose-dependent manner. Interestingly, in Saos-2 cells, a lower ratio of Δ40-p53/full-length p53 increased transcriptional activity over full-length p53 alone, suggesting that this interaction varies upon cellular context. In these studies, full-length p53 was also protected from degradation by MDM2 when co-transfected with Δ40-p53.[59] Although splice variants in p53 that alter the C-terminal domain require more investigation, p53β has been shown to display promoter-specific binding capabilities.[48]

Given functional differences among p53 splice variants, deregulation of these variants in cancer has come under scrutiny. Mutations affecting *TP53* splicing are found in many different

cancers, with some of these splice variants previously considered to be 'neutral' as they do not change amino-acid composition.[60] In a reverse transcriptase–PCR analysis of pooled RNA from normal breast tissue, all three C-terminal splice variants could be detected, but Δ133-p53 variants were absent. In the same analysis on RNA extracted from a panel of 30 breast tumors, only 5 of which were identified to harbor p53 mutations, p53γ was not observed, whereas Δ133-p53α was found in 24 samples.[48] In a separate cohort of 127 breast tumors, only 19% expressed both p53β and p53γ. By comparing tumors with p53 mutations, patients with concomitant loss of p53γ showed poor survival, whereas those who co-expressed p53γ had lower rates of recurrence, with survival similar to patients with functional wild-type p53.[61] Both Δ40-p53 and p53β are expressed in a majority of melanoma cell lines and primary isolates of metastatic melanoma, but are absent or expressed at low levels in fibroblasts and melanocytes. Δ133-p53β is the only other splice variant expressed in melanoma cell lines, but at very low levels.[62]

In addition to the previously described N-terminal and C-terminal splice variants, a panel of 34 ovarian cancer cell lines revealed that internal exon skipping of *TP53* can also occur. This was validated in a cohort of 245 primary ovarian cancers, with expression of the splice variants lower than observed in the cell lines. P53δ associated with decreased recurrence free and overall survival, supporting the idea that splice-site variants and nonsynonymous mutations can be similarly detrimental. Concomitant expression of p53β and functional wild-type p53 also correlated with decreased survival.[53] In a separate study, Δ133-p53 and Δ40-p53 isoforms were identified in both stages I and stage III ($n = 83$ and 86, respectively) ovarian cancers. Neither was associated with malignant progression, although the authors could not rule out a role in tumor initiation.[63]

Overall, it appears that the N-terminal and C-terminal splice variants of *TP53* have distinct effects in cancer. The absent TAD in N-terminal variants provides an apparent mechanism to inactivate p53, and perhaps unsurprisingly, has been identified repeatedly in cancer. C-terminal variants require further investigation, however, some of these isoforms appear to be functional, given that expression of p53γ alongside mutant p53 phenocopies the survival of patients with wild-type p53.[61]

BRCA1-ASSOCIATED RING DOMAIN 1 (*BARD1*)

A majority of women who inherit mutations in the *BRCA1* breast cancer susceptibility gene will develop breast cancer[64] and 39% will develop ovarian cancer.[65] The search for potential binding partners of this tumor suppressor led to identification of *BARD1*,[66] a gene that spans 11 exons and encompasses nearly 85 kb on chromosome 2. Both BRCA1 and BARD1 have similar protein structures and form a heterodimer by binding through their RING finger domains (Figure 4a). This domain contains critical residues that are mutated in *BRCA1* in association with breast and ovarian tumors. These data suggest that interactions between BRCA1 and BARD1 are required for tumor suppression.

Given this critical interaction, it is surprising that *BRCA1* shows frequent mutations (1653 as surveyed by the HGMD[67]), whereas *BARD1* mutations in both familial and sporadic breast cancer are relatively rare.[67–70] *BARD1* is more than just an accessory protein for *BRCA1*; the two genes are not co-expressed universally in all tissues,[71] and in response to genotoxic stress, *BARD1* functions in a *BRCA1*-independent manner to induce p53-dependent apoptosis.[72] In addition, homozygous loss of *BARD1* in mice results in an embryonic lethal phenotype, similar to *BRCA1*-null mice.[73] Indeed, *BARD1* itself has come to be recognized as a potent tumor suppressor.[74]

The relative paucity of *BARD1* mutations is especially puzzling in light of several genome-wide association studies that identified *BARD1* as a susceptibility gene for neuroblastoma.[75–77] Among the first clues to this puzzle was the observation of aberrant *BARD1* splicing in NuTu-19 cells, a rat ovarian cancer cell line[78] that does not express full-length *BARD1*. Reintroduction of the full-length protein induced apoptosis, suggesting that this splice variant is defective in tumor suppression. Lacking the RING domain, this variant (*BARD1δ*, Figure 4b) is also expressed in several breast cancer lines, and co-immunoprecipitation experiments have shown that it is unable to interact with *BRCA1*.[79]

Could aberrant *BARD1* splicing also confer dominant-negative regulation or other oncogenic properties? This scenario is suggested by the finding that BARD1 overexpression is associated with poor survival in breast and ovarian tumors.[80] That BARD1 expression is inversely associated with outcome contrasts its purported role as a tumor suppressor. In addition, although the protein had been described as nuclear,[66,81] non-apoptotic cells exhibit cytoplasmic localization. Analysis at the RNA level revealed that many of these transcripts have N-terminal truncations and lead to decreased protein stability.[82]

An additional survey of cervical, breast, ovarian and endometrial cancer cell lines, along with ovarian tumor tissue arrays revealed that *BARD1* spliced isoforms are often more abundant than the full-length product.[83] This survey also identified and enumerated splice isoforms *BARD1α-η* (Figure 4b), as well as alternative transcriptional start sites in exon 4 (Ω1, Ω2, Ψ) (Figure 4c). RNA interference experiments directed only toward the full-length transcript did not affect cell growth, whereas small interfering RNAs targeting both full-length and *BARD1* splice isoforms reduced proliferation, suggesting that *BARD1* splice variants have functional roles.[83,84] Indeed, while full-length BARD1 normally promotes Aurora B degradation, BARD1β opposes this and promotes proliferation by scaffolding Aurora B and BRCA2 in late-stage mitosis in a BRCA1-independent manner.[84] BARD1δ also has antagonistic functions relative to full-length BARD1. In MCF7 cells, the BRCA1-BARD1 complex leads to estrogen receptor alpha ubiquitination and degradation, but overexpression of BARD1δ instead enhances estrogen receptor alpha stability and transcriptional activity.[85]

Up to 19 different splice isoforms of *BARD1* have been identified in colon cancer[86] with a concomitant lack of full-length BARD1 expression.[87] Furthermore, in analyses of human tumor biopsies with BARD1 epitope-specific immunohistochemistry, patients with expression of BARD1κ and BARD1π, both presumed to be internal deletion mutants, as well as expression of BARD1β showed poor outcome in non-small cell lung cancers.[88] As aberrant splicing of *BARD1* is not limited to hormonally regulated tumors, could the susceptibility locus in neuroblastoma demonstrate aberrant splicing as well? Analysis of *BARD1* isoforms cloned from neuroblastoma cell lines and fetal sympathetic ganglia characterized a total of 15 unique splice variants in addition to the full-length transcript.[89] In particular, expression of *BARD1β* was associated with the homozygous GG neuroblastoma risk allele at rs6435862. Similar to observations in ovarian cancer, knockdown and overexpression experiments in neuroblastoma cell lines reiterated the oncogenic characteristics of *BARD1β*.[89]

It remains unclear why *BARD1* loss-of-function in cancer proceeds predominantly through alternative splicing as opposed to mutation. *BARD1* has functions outside of the *BRCA1* signaling axis, and mutations within *BARD1* may be too deleterious for cancerous cells to overcome. In this case, alternative splicing could provide a mechanism in which activity is finely modulated, rather than abolished. The distinction between the loss of a tumor suppressor and the activation of oncogenic properties can be difficult. It is also possible that these splice variants gain oncogenic BRCA1-independent functions. The effects of *BARD1* alternative splicing in neuroblastoma also demonstrate the importance of evaluating alternative splicing in conjunction with mutational surveys when

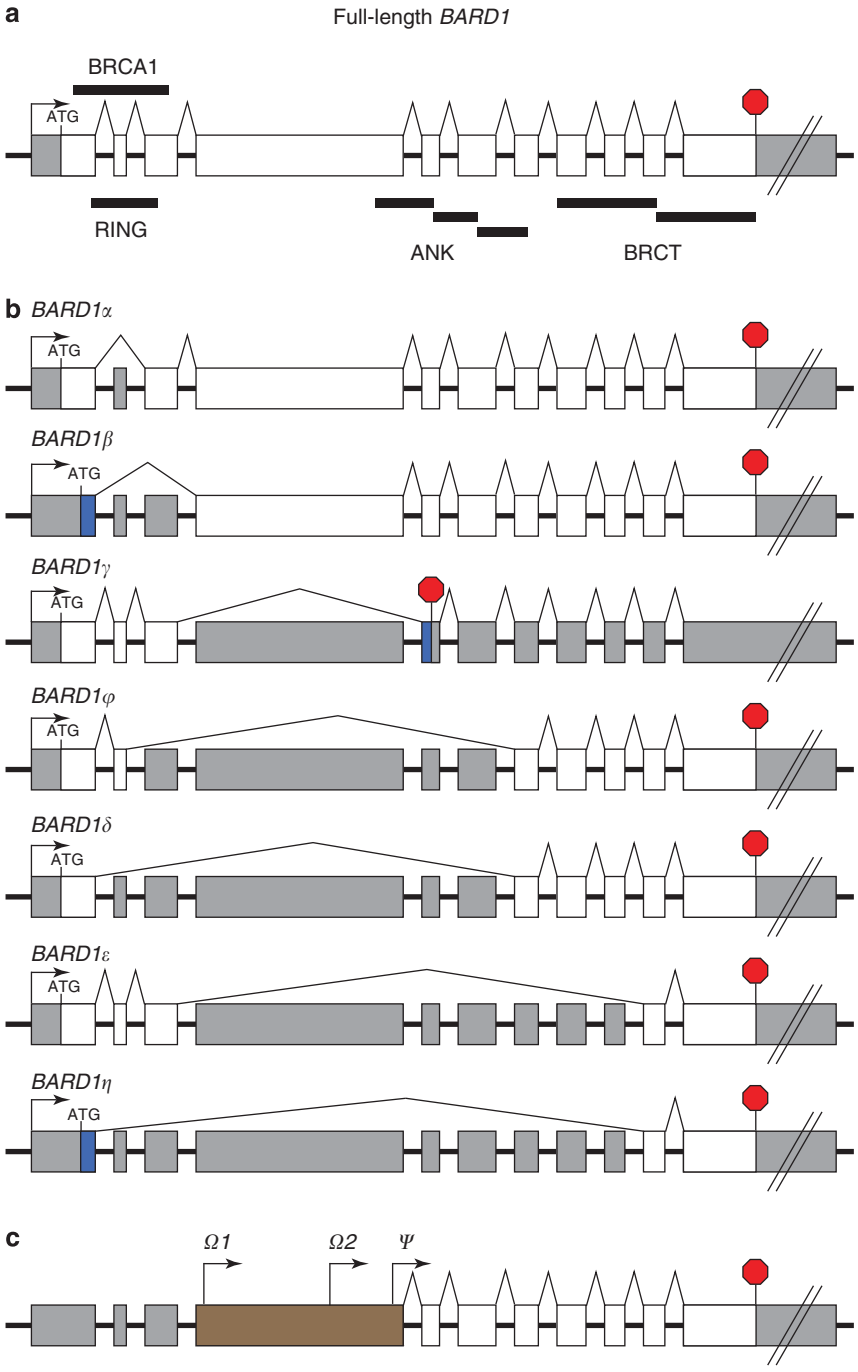

Figure 4. Splicing schematic of *BARD1*. (**a**) Exon structure of full-length *BARD1* with relative locations encoding the RING domain, ANK repeats and BRCT domains (as described in UniProt). BRCA1 and BARD1 interact through their respective ring domains. Exon sizes are relative to actual length, but introns are not drawn to scale. Start and stop codons are located at the indicated positions. Coding sequences are indicated as white-filled boxes, and untranslated regions are indicated as gray-filled boxes. (**b**) *BARD1* splice variants exhibit various combinations of alternative polyadenylation and exon skipping. Alternative reading frames are depicted in blue. (**c**) Alternative promoters were found in exon 4 (brown) and encode for varying translational start methionines.

considering loci and genes identified through genome-wide association studies or quantitative-trait loci mapping experiments.

ANDROGEN RECEPTOR

Signaling through the androgen receptor (*AR*), a steroid hormone receptor transcription factor,[90] is critical for prostate cancer development.[91,92] Ligand binding triggers receptor homodimerization and nuclear translocation, which in turn executes a transcriptional growth program through binding specific AR element sequences.[93] *AR* spans approximately 200 kb on Xq11–12 and has eight exons that encode the full-length 110 kDa protein.[94] Exons 2 and 3 encode most of the DNA-binding domain[95] and exons 4–8 encode the ligand-binding domain[96] (Figure 5a). A naturally occurring splice variant of *AR* exists and consists of an alternative first exon. This transcript is found in various tissues, with robust expression in the heart.[97] Although the canonical first exon constitutes over 50% of the full-length transcript, this alternative N-terminal

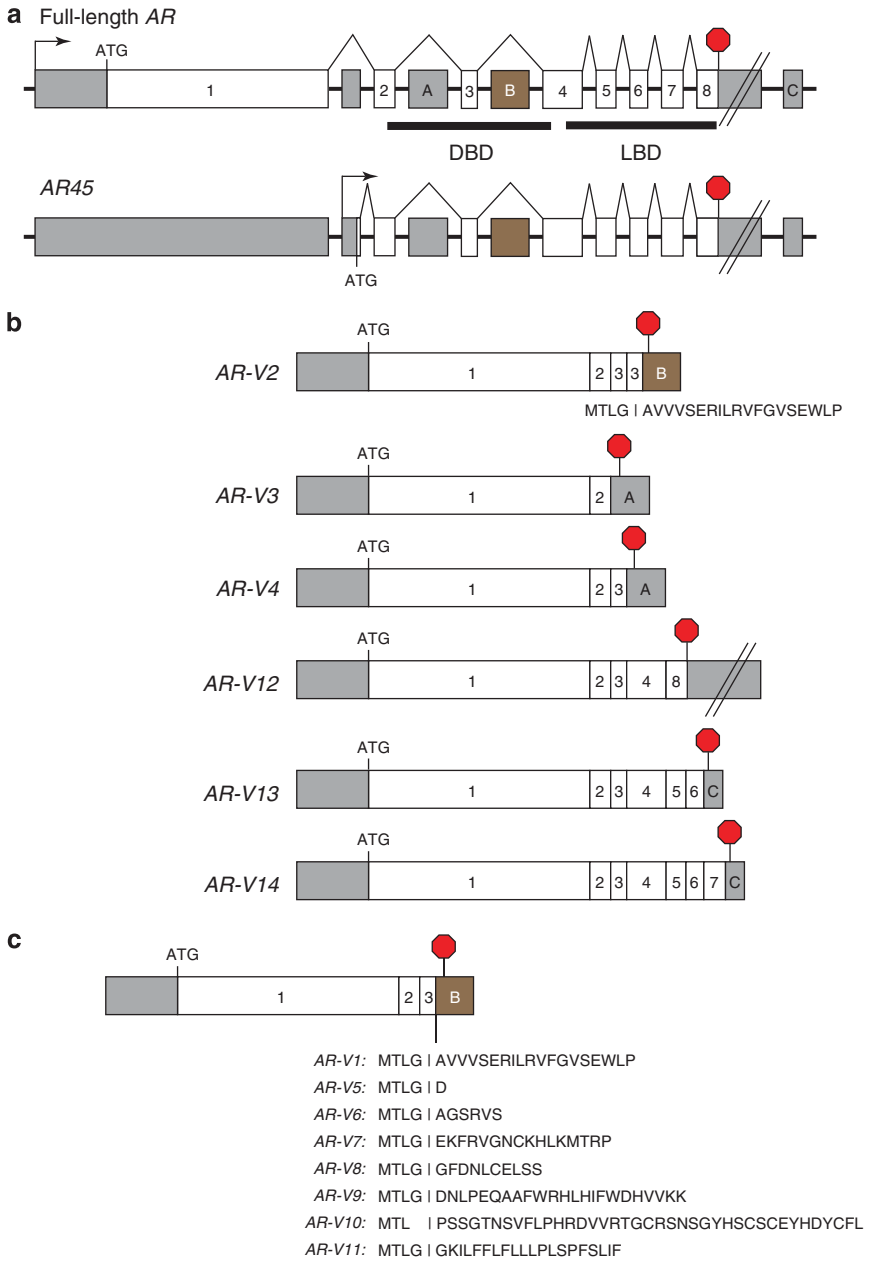

Figure 5. Splicing schematic of *AR*. (**a**) Exon structures of full-length *AR* and the canonically spliced *AR45* variant. Relative locations encoding the DNA-binding domain (DBD) and ligand-binding domain (LBD) are indicated (as described in UniProt). Exon sizes are relative to actual length, but introns are not drawn to scale. Start and stop codons are located at the indicated positions. Coding sequences are indicated as white-filled boxes, and untranslated regions are indicated as gray-filled boxes. Exons are numbered as they appear in full-length *AR*. Exon A has been previously described as Cryptic Exon 4 (CE-4), and exon C has been previously described as exon 9. Region B (brown) includes the previously described CE-2, CE-3, CE-4, CE-5 and various cases of intron retention. (**b**) Aberrant splicing of *AR* can include exon scrambling (*AR-V2* and *AR-V4*) as well as exon skipping (*AR-V12*–*AR-V14*). The translated amino-acid sequence for the truncated C-terminus in region B is given for *AR-V2* starting with the end of exon 3. (**c**) *AR* splice variants often include intron retention or the splicing of cryptic exons and subsequent truncation after exon 3. The translated amino-acid sequence for the truncated C-terminus in region B is given for the splice variants starting with the end of exon 3.

domain contains only seven amino acids and was shown to inhibit proliferation when overexpressed in LNCaP cells (Figure 5b). Dubbed AR45 due to its calculated 45 kDa molecular weight, this isoform forms heterodimers with full-length AR in a ligand-dependent manner and inhibits AR activity, presumably due to an inability of the alternative N-terminal domain to recruit a full set of transcriptional coactivators.[97–99]

Androgen deprivation therapy has formed the backbone of prostate cancer therapy for over 70 years,[100] achieved through surgical castration or drugs that block androgen biosynthesis.[101,102] Recurrent disease arises when the tumor loses androgen sensitivity, termed castration-resistant prostate cancer (CRPC). Comparative genomic hybridization revealed that amplification of *AR* may be utilized by CRPC to promote growth in low androgen conditions.[103] The fact that normal *AR* isoforms show functional differences based on alternative splicing suggests another mechanism that could be exploited by CRPC. In fact, androgen insensitivity syndrome, a developmental disorder

resulting in the impaired development of internal and external male phenotypes, is due to compromised androgen signaling.[104] Interestingly, aberrant splicing of *AR* has been found in both partial and complete androgen insensitivity syndrome, resulting in receptors unable to bind ligand, as well as receptors that have no transcriptional activity even after ligand binding.[105–109]

All of these previously described alternatively spliced variants result in loss-of-function in AR. Might gain-of-function in AR isoforms be advantageous in CRPC? An AR isoform lacking the ligand-binding domain was first identified in the prostate cancer cell line 22Rv1[110] and subsequently detected in prostate cancer tumor tissue.[111] This isoform could act independently of ligand and was more efficient than full-length AR in activating transcription. Although this isoform was originally thought to result from proteolytic cleavage, knockdown experiments indicated that the truncated isoform arises because of alternative splicing. RNA interference targeting an exon partly encoding the ligand-binding domain only eliminated full-length AR, whereas small interfering RNAs targeting exon 1 knocked down all AR isoforms. In fact, the truncated isoform was found to use an alternative exon 2,[112] which excluded post-translational modifications as a mechanism to generate this isoform. Further analysis of the 22Rv1 line has since revealed several other *AR* splice variants.[113]

Computational methods have identified three cryptic exons located between exons 3 and 4 and led to discovery of one of the best-characterized splice variants, *AR-V7* (Figure 5b). Also cloned from 22Rv1 cells, this transcript contains a cryptic exon adjoined to exon 3 that harbors a stop codon after 17 amino acids.[114,115] Expression of this transcript was increased by ~20-fold when comparing CRPC with hormone naive prostate cancer. A subset of the hormone naive samples with high expression levels indicated that high *AR-V7* expression was a strongly associated with recurrence following surgical treatment.[114] Expression of AR-V7 was examined by immunohistochemical analysis of 429 human prostate tissue samples, where it was also found to be upregulated in CRPC as compared with hormone naive samples. A higher cytoplasmic staining score also correlated with increased risk of recurrence after surgery.[115] AR-V7 was constitutively active, with isoform-specific knockdown resulting in reduced proliferation *in vitro* and *in vivo*.[114,115]

An additional novel *AR* splice variant, identified from a human bone metastasis, contains a partially retained intron between exons 2 and 3 that disrupts the DNA-binding domain.[116] The LuCaP xenografts, 25 prostate cancers mostly derived from CRPC metastases, identified another splice variant lacking exons 5–7.[117] Other cryptic exons, including one downstream of exon 8, were discovered in two CRPC samples using a custom tiling microarray that spanned 200 kb and encompassed the *AR* locus.[118] Furthermore, a separate immunohistochemical tissue microarray analysis of 50 primary prostate cancers and 162 metastatic CRPC samples, using AR antibodies specific either for the N- or C-terminus, noted an increase of AR variants in CRPC but not primary prostate cancer.[119]

Additional *AR* splice variants have also been profiled in the VCaP (human) and Myc-CaP (murine) cell lines using deep sequencing methods.[120] Interestingly, these variants were expressed at very low levels relative to full-length *AR* (~0.1–2.5%) and gain-of-function was dependent on expression of full-length *AR*. Although these data suggest that *AR* splicing is inconsequential in these models, subsequent RNA interference experiments targeting full-length *AR* while measuring the activity of AR-V7 in the presence or absence of the AR agonist R1881 showed no difference.[118] As constitutively active splice variants can be expressed in the absence of full-length *AR*,[117] it appears that cellular context is critical to modulate the activity of conditionally active splice variants. Further functional analysis of truncated AR variants showed that C-terminal variation leads to differences in nuclear localization. However, regardless of the specific C-terminus, AR isoforms possess a basal level of nuclear localization and ligand independent, constitutive transcriptional activity. Indeed, the transcriptional ability of each individual variant is promoter dependent and is not affected by mutations that alter nuclear localization.[121]

Ultimately, the complex interplay between AR and its splice variants requires further investigation, but the abundance of aberrant splicing after exon 3 also adds to the intrigue of *AR* splicing. Transcripts involving out of order sequences (*AR-V3* and *AR-V4*, Figure 5b) have been identified, but are these splice variants examples of exon scrambling,[122,123] or are there underlying genomic structural variations? This region remains a prime candidate to examine genetic control of splicing given the abundance of cryptic exons and diverse examples of intron retention (Figure 5c).

THE SPLICING PATHWAY

The serine/arginine rich (SR) family of proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) were among the first to be identified to regulate alternative splicing[124,125] and remain the best-studied *trans*-acting splicing factors. SR proteins typically facilitate splice site recognition and promote inclusion of alternatively spliced exons by binding to regulatory sequences present in the pre-mRNA, intronic and exonic splicing enhancers (Figures 6a and b). Upon binding, they can interact with other splicing co-activators or directly recruit spliceosomal small nuclear ribonucleoproteins to the splice site and activate splicing.[126,127] HnRNPs can oppose this action by binding to intronic and exonic splicing silencers on the pre-mRNA and inhibit recognition of the splice site (Figures 6a and c).[3,128] These splicing inhibitors are generally thought to sterically hinder spliceosomal small nuclear ribonucleoproteins or other splicing enhancers from binding, although additional mechanisms have been proposed to contribute to exclusion of alternatively spliced exons (reviewed in Chen and Manley[3]). Ultimately, whether an exon is included or excluded from the final mRNA transcript is determined by the relative concentrations of the activators and repressors at the splice site.[129,130]

Although the cause of most aberrant splicing events remains unknown, an understanding of known splicing factors serves as a starting point for understanding mechanism. SF2/ASF, encoded by the gene *SFRS1*, is an SR protein that is also essential for genomic stability.[131] Overexpression of this gene in a large panel of lung, colon and breast tumors identified *SFRS1* as a proto-oncogene,[132] and its transforming abilities have been shown to act through the mTOR Complex 1 signaling axis.[133] Downregulation of another SR family member, *SRSF3*, promotes alternative splicing of p53, resulting in an increase of p53β and downstream cellular senescence.[134]

The transcription of several hnRNP proteins, including the polypyrimidine tract binding protein, is activated by the MYC oncogene. In cancer, these splicing factors bind to and inhibit inclusion of exon 9 for *PKM*, promoting the embryonic PKM2 isoform and aerobic glycolysis.[135] Similarly, transcriptional upregulation of hnRNPA1 is downstream of EGFRvIII in glioma, and it affects alternative splicing of *MAX* to produce ΔMax and promote glycolytic gene expression and proliferation in glioma cells.[136] HnRNPA1 deregulation is also prominent in lung, colon and renal cancers.[137–139] Another hnRNP family member, hnRNPH, is overexpressed in glioblastoma where it may promote malignant progression by switching the splicing preferences of *RON* and *MADD*. *RON* encodes a receptor tyrosine kinase and is spliced into a ligand-independent, constitutively active form that promotes invasion and metastasis. *MADD* encodes a death domain containing adaptor protein that mediates apoptotic tumor

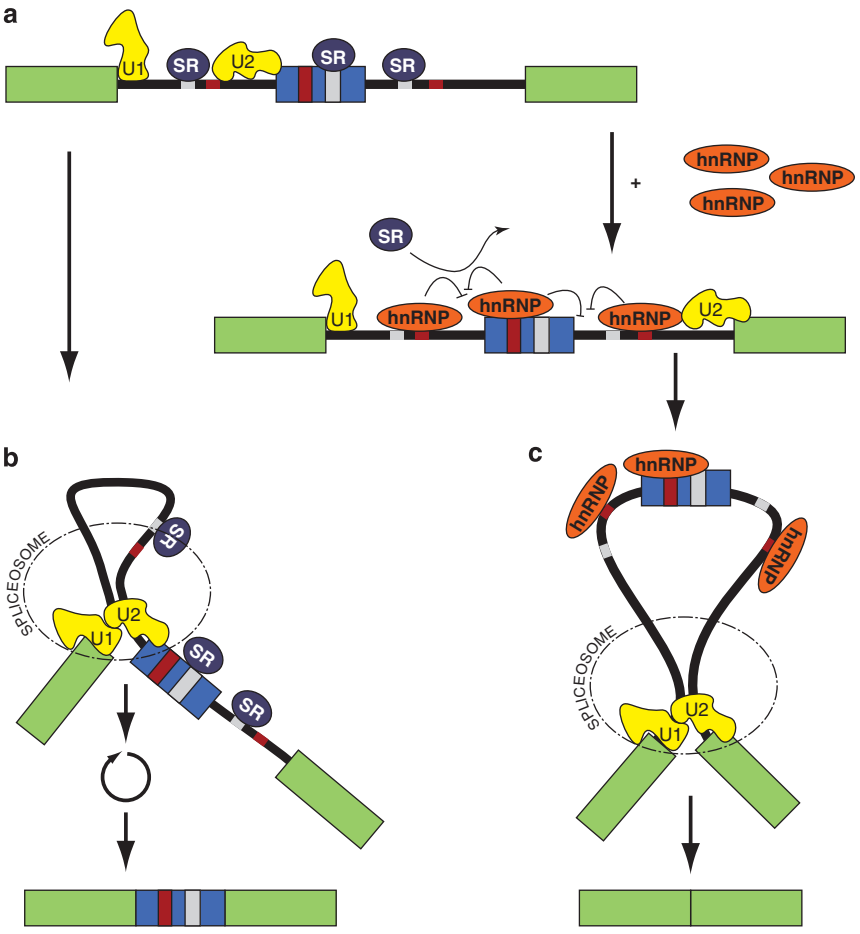

Figure 6. Genomic regulation of splicing. The decision to splice an alternative exon (blue) with constitutively expressed exons (green) is largely governed by the concentration of accessory splicing factors that recognize and bind splicing motifs. (**a**) SR proteins promote exon inclusion by binding intronic splicing enhancers (ISEs) or exonic splicing enhancers (ESEs) (gray) on the pre-mRNA and recruit spliceosomal small nuclear ribonucleoproteins (snRNPs—yellow) to the splice site. Other spliceosomal snRNPs are also recruited to splice the alternative exon, with successive rounds of splicing at downstream exons as in **b**. This can be opposed by heterogeneous nuclear ribonucleoproteins (hnRNPs) that recognize intronic splicing silencers (ISSs) or exonic splicing silencers (ESSs; red) on the pre-mRNA and inhibit SR protein binding or spliceosomal snRNPs from recognizing the splice site. The downstream splice site is recognized instead, and the alternative exon is excised with the rest of the intron as in **c**.

necrosis factor-α signaling, and it is spliced into an antagonistic anti-apoptotic variant.[140]

Although these two protein families are perhaps the most ubiquitously expressed and well-studied splicing factors, others have also been found to contribute to tumorigenesis. For example, *CD44* is known to undergo complex alternative splicing involving 10 variant exons,[141] and in particular, exon v5 is critical for promoting metastasis.[142] The transcriptional co-activator SND1 is also an effector of alternative splicing, as it interacts with the splicing factor SAM68 to promote *CD44* exon v5 inclusion. Both SND1 and SAM68 are upregulated in prostate cancer, and knockdown of SND1 or SAM68 reduces proliferation and migration of prostate cancer cells.[143]

The splicing landscape as a whole has also come under recent scrutiny in cancer. Exon array analysis of a cohort of 47 neuroblastoma patients revealed numerous splicing differences separating stage 1 and high-risk stage 4 disease, including *PKM*, *KIF1B* and *MAP2*.[144] Similarly, exon arrays have been used to examine alternative splicing in 102 normal and cancer tissue samples from colon, urinary bladder and prostate cancer. Over 2000 candidate alternative splicing events distinguished tumor and normal tissues, with a few genes (*ACTN1*, *CALD1* and *VCL*) found in all three tumor tissues.[145] In addition, whole-exome sequencing of 29 myelodysplasia specimens, a frequent precursor of acute myeloid leukemia, identified frequent mutations in the splicing pathway.[146]

ALTERNATIVE SPLICING AND CANCER THERAPY

The tumorigenic advantages provided by alternative splicing are not limited to proliferation or apoptotic blockade. Aberrant splicing can also provide a means for therapeutic evasion by gain-of-function. As we improve our understanding of the molecular basis of different cancers, the development of highly specific small molecule inhibitors has yielded tremendous success.[147] Once cancer is detected in the clinic and treatment commences, selective pressures within a tumor typically promote therapy resistance. Almost 80% of patients enrolled in a phase I clinical trial for metastatic melanoma observed partial or complete response to vemurafenib, an inhibitor of BRAF(V600E).[148] Invariably, patients relapse, developing resistance through a number of mechanisms.[149–151] Interestingly, in an *in vitro* model of inhibitor resistance, a novel splice variant lacking exons 4–8 has promoted BRAF dimerization and vemurafenib-resistant extracellular signal-regulated kinase signaling. Six of 19 patients with acquired resistance to vemurafenib also displayed BRAF(V600E) splice variants, indicating that therapy resistance could also be achieved through aberrant splicing.[152]

With emerging data associating splice variants with survival, can this information be used to improve therapy? If aberrant splicing leads to functional consequences, would correcting the splicing, perhaps in conjunction with conventional therapy, improve survival in cancer? A mouse model of spinal muscular atrophy provides clues in this regard. Spinal muscular atrophy is a genetic disease where loss of the SMN protein causes death of motor neurons in the anterior horn of the spinal cord, resulting in progressive and debilitating weakness. Although caused by loss-of-function mutations in *SMN1*,[153] disease severity is modulated by the paralog *SMN2*, which is able to produce varying levels of functional protein. The variability in expression of functional *SMN2* stems from the fact that exon 7 is predominantly skipped, resulting in a nonfunctional SMN.[154] Anti-sense oligonucleotides targeting an intronic splicing silencer have been used to correct *SMN2* splicing and restore SMN expression, providing effective long-term rescue of spinal muscular atrophy in mice.[155] If pharmacologic anti-sense oligonucleotides can be developed to achieve specific knockdown *in vivo*, this approach could be directed to tumor-specific splice variants for cancer therapy.

As the pharmacologic development of anti-sense oligonucleotides continues, conventional therapy remains driven by small-molecule inhibitors and derivatives of naturally occurring compounds with antitumor effects. Herboxidiene,[156] FR901464[157] and the pladienolides[158] are natural products that modulate spliceosomal components. These were of particular interest because of their low nanomolar IC_{50} and cytotoxic effects in cancer cell lines and animal models. They have led directly to the development of synthetic derivatives: E7107 from pladienolide B, spliceostatin A and meayamycin from FR901464, and the sudemycins, a set of molecules designed using a consensus pharmacophore gleaned from known structure-activity relationships (reviewed in Bonnal *et al.*[159] and Webb *et al.*[160]). SF3B, a subunit of the U2 small nuclear ribonucleoprotein that facilitates splice site recognition (Figure 6), is the main target of these compounds,[161,162] and they modulate not only alternative splicing, but also expression of genes critical to cancer progression.[163–165]

By effecting intron retention or exon skipping, these drugs ultimately lead to activation of the nonsense-mediated decay pathway or the production of inactive cell cycle genes, resulting in cellular arrest.[161,162,166] Interestingly, these drugs show tumor-specific effects with little toxicity, despite theoretical deleterious effects on normal cells. One plausible explanation is that drug dosage is critical to avoid complete inhibition of splicing, which would be universally toxic. At an appropriate dose, these drugs may still be effective at eliminating aberrant splicing or minimizing the splice isoforms that are critical for cancer progression. In this setting, cancers that have acquired deregulation of global splicing patterns may be best suited for spliceosome-inhibitory therapy. It is also possible that tumors driven in-part through functionally aberrant splicing may be exquisitely sensitive to these spliceosomal modulators, as the drugs reverse the splicing dependency. Future studies analyzing the molecular underpinnings of these drugs may reveal more about the functional roles of alternative splicing in cancer.

CONCLUSIONS AND PERSPECTIVES

The overexpression experiments in p53-null cells that revealed the dose-dependent dominant-negative regulatory effects of Δ40-p53[59] also serve to highlight the importance of the ratio between splice variants. Is there an absolute threshold for expression of any particular splice isoform to produce a biological effect? The answer is certainly transcript-specific, as the normal function of the full-length product is just as critical as the distinction of the novel splice isoforms as gain-of-function or dominant-negative regulators. All three genes discussed here suggest that cellular context is also an important factor, and as evidenced by the inconsistent data between splice isoform ratios in *AR*, the answer is not always clear. Furthermore, minor amounts of aberrant splice isoforms may be indicative of global splicing deregulation with combinatorial effects in cancer. These issues may be better addressed as technology continues to improve in detecting splice isoforms and quantitating their expression.

Despite the uncertainty in this regard, the influence and impact that alternative splicing has in cancer is evident in the association of splice variants with outcome and the profound effects that splicing can have on therapy. Although interest in cancer-associated splicing continues to increase, the specific regulatory mechanisms harnessed to confer tumorigenic advantages are still poorly understood. This is true regardless of whether these events occur through aberrant splicing or modulation of existing alternative splicing.

Large-scale, unbiased proteomic approaches have been used to identify additional splicing factors.[167] However, given the tissue- and cellular-specificity of splicing programs, can these methods be truly comprehensive? Along these same lines, splicing motifs have been so far defined by various methods. SELEX (systematic evolution of ligands by exponential enrichment) has identified exonic splicing enhancers *in vitro*[168,169] and *in vivo*,[170] but this method necessitates a known splicing factor to begin. Computational methods have also identified exonic splicing enhancers[171,172] and intronic splicing enhancers[173,174] utilizing several approaches including motif comparisons between introns and exons, exons with weak splice sites and exons with strong splice sites, and evolutionary conservation. An unbiased approach using a splicing reporter assay to analyze all possible decamer motif combinations identified 109 intronic splicing enhancers.[175] The fact that some of these motifs overlapped with previously identified exonic splicing silencers indicates the importance of positional effects on motif function. This assay utilized a reporter system in which random decamers were cloned into a specific position, but as human introns extend an average of 3.7 kb,[176] a truly unbiased approach including positional effects remains to be examined.

The importance of defining and understanding these motifs should not be understated. The genetics of splicing clearly have crucial roles in cancer, as exemplified by the common variation associated with *BARD1* splicing and the splice site mutations demonstrated in both *TP53* and *AR*. Current technologies provide a wealth of sequencing data that has revolutionized the way we analyze mutations in cancer. However, mutational effects on splicing are typically ignored when distinguishing synonymous from nonsynonymous mutations. Similarly, a mutation that ultimately results in a premature stop codon often leads the resulting transcript and protein to be automatically classified as non-functional, without regard to additional splice variants. For example, some of the *TP53* splicing experiments were performed in the K562 cell line, which has been categorized as 'p53 null' because of a premature stop codon at residue 148. These cells still expressed the Δ160-p53 isoform, indicating that functional splice variants are still being produced from transcripts carrying frame-shift or nonsense mutations.[177]

In addition, high-throughput sequencing in cancer primarily focuses on the exome, mostly due to the benefits of the accompanying increase in coverage depth. Although these studies clearly yield insights into the genetics of disease, changes in splicing can easily be missed by failing to capture regulatory regions within the introns. It could be argued that as we do not have a definition for precise motifs at specific intronic positions to look for, there is no justification for whole-genome sequencing. Yet is there a better way to understand the genetic regulation of splicing without these data?

Next-generation precision medicine therapies are on the horizon and promise highly specific, perhaps even isoform specific, targeting abilities. In-depth genomic studies of splicing

will be critical for defining splicing mutations that occur away from intron–exon boundaries. These data, coupled with biochemical and molecular analyses using splicing-modulating drugs, promise to uncover new targets in cancer and to enable novel approaches to block canonical cancer targets and pathways.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to Clay Gustafson, Miller Huang and Erin Simonds for reviewing the manuscript. The Weiss lab is supported by NIH grants CA176287, CA82104, CA133091, CA102321, CA148699, CA159859, CA163155 and CA081403; and the CureSearch, Katie Dougherty, Pediatric Brain Tumor, St Baldricks and Samuel G Waxman Foundations.

REFERENCES

1 Pan Q, Shai O, Lee L, Frey B, Blencowe B. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet* 2008; **40**: 1413–1415.
2 Burge CS, Tuschl T, Sharp PA. Splicing of precursors to mRNAs by the spliceosomes. In: Gesteland RF, Cech TR, Atkins JF (eds) *RNA World*, Second edn. Cold Spring Harbor Laboratory Press: Woodbury, NY, 1999, pp 525–560.
3 Chen M, Manley J. Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches. *Nat Rev Mol Cell Biol* 2009; **10**: 741–754.
4 Wahl M, Will C, Lührmann R. The spliceosome: design principles of a dynamic RNP Machine. *Cell* 2009; **136**: 701–718.
5 Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. *Nat Rev Genet* 2010; **11**: 345–355.
6 Ellis JD, Barrios-Rodiles M, Çolak R, Irimia M, Kim T, Calarco JA *et al.* Tissue-specific alternative splicing remodels protein-protein interaction networks. *Mol Cell* 2012; **46**: 884–892.
7 Wu J, Habegger L, Noisa P, Szekely A, Qiu C, Hutchison S *et al.* Dynamic transcriptomes during neural differentiation of human embryonic stem cells revealed by short, long, and paired-end sequencing. *Proc Natl Acad Sci USA* 2010; **107**: 5254–5259.
8 Kalsotra A, Cooper TA. Functional consequences of developmentally regulated alternative splicing. *Nat Rev Genet* 2011; **12**: 715–729.
9 Heyd F, Lynch K. DEGRADE, MOVE, REGROUP: signaling control of splicing proteins. *Trends Biochem Sci* 2011; **36**: 397–404.
10 Xu Q. Discovery of novel splice forms and functional analysis of cancer-specific alternative splicing in human expressed sequences. *Nucleic Acids Res* 2003; **31**: 5635–5643.
11 Hui L, Zhang X, Wu X, Lin Z, Wang Q, Li Y *et al.* Identification of alternatively spliced mRNA variants related to cancers by genome-wide ESTs alignment. *Oncogene* 2004; **23**: 3013–3023.
12 Venables J, Klinck R, Koh C, Gervais-Bird J, Bramard A, Inkel L *et al.* Cancer-associated regulation of alternative splicing. *Nat Struct Mol Biol* 2009; **16**: 670–676.
13 Boise L, González-García M, Postema C, Ding L, Lindsten T, Turka L *et al.* Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* 1993; **74**: 597–608.
14 Xerri L, Parc P, Brousset P, Schlaifer D, Hassoun J, Reed J *et al.* Predominant expression of the long isoform of Bcl-x (Bcl-xL) in human lymphomas. *Br J Haematol* 1996; **92**: 900–906.
15 Takehara T, Liu X, Fujimoto J, Friedman SL, Takahashi H. Expression and role of Bcl-xL in human hepatocellular carcinomas. *Hepatology* 2001; **34**: 55–61.
16 Bouillet P, O'Reilly LA. CD95, BIM and T cell homeostasis. *Nat Rev Immunol* 2009; **9**: 514–519.
17 Cascino I, Fiucci G, Papoff G, Ruberti G. Three functional soluble forms of the human apoptosis-inducing Fas molecule are produced by alternative splicing. *J Immunol* 1995; **154**: 2706–2713.
18 Cheng J, Zhou T, Liu C, Shapiro JP, Brauer MJ, Kiefer MC *et al.* Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. *Science* 1994; **263**: 1759–1762.
19 Liu JH, Wei S, Lamy T, Li Y, Epling-Burnette PK, Djeu JY *et al.* Blockade of Fas-dependent apoptosis by soluble Fas in LGL leukemia. *Blood* 2002; **100**: 1449–1453.
20 Sheen-Chen S-M, Chen H-S, Eng H-L, Chen W-J. Circulating soluble Fas in patients with breast cancer. *World J Surg* 2003; **27**: 10–13.
21 Kondera-Anasz Z, Mielczarek-Palacz A, Sikora J. Soluble Fas receptor and soluble Fas ligand in the serum of women with uterine tumors. *Apoptosis* 2005; **10**: 1143–1149.
22 Warburg O. On the origin of cancer cells. *Science* 1956; **123**: 309–314.
23 Noguchi T, Inoue H, Tanaka T. The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. *J Biol Chem* 1986; **261**: 13807–13812.
24 Mazurek S, Boschek C, Hugo F, Eigenbrodt E. Pyruvate kinase type M2 and its role in tumor growth and spreading. *Semin Cancer Biol* 2005; **15**: 300–308.
25 Christofk HR, vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R *et al.* The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 2008; **452**: 230–233.
26 David CJ, Manley JL. Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. *Genes Dev* 2010; **24**: 2343–2364.
27 Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. *Nature* 1979; **278**: 261–263.
28 Linzer D, Levine A. Characterization of a 54K Dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* 1979; **17**: 43–52.
29 Finlay C, Hinds P, Levine A. The p53 proto-oncogene can act as a suppressor of transformation. *Cell* 1989; **57**: 1083–1093.
30 Baker SJ, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 1989; **244**: 217–221.
31 Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer* 2009; **9**: 749–758.
32 Fields S, Jang SK. Presence of a potent transcription activating sequence in the p53 protein. *Science* 1990; **249**: 1046–1049.
33 Raycroft L, Wu HY, Lozano G. Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. *Science* 1990; **249**: 1049–1051.
34 Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* 1991; **352**: 345–347.
35 Shaw P, Bovey R, Tardy S, Sahli R, Sordat B, Costa J *et al.* Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. *Proc Natl Acad Sci USA* 1992; **89**: 4495–4499.
36 Serrano M, Lin A, McCurrach M, Beach D, Lowe S. Oncogenic Ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 1997; **88**: 593–602.
37 Wang Y, Blandino G, Oren M, Givol D. Induced p53 expression in lung cancer cell line promotes cell senescence and differentially modifies the cytotoxicity of anti-cancer drugs. *Oncogene* 1998; **17**: 1923–1930.
38 Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K *et al.* A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* 2000; **404**: 42–49.
39 Vogelstein B, Lane D, Levine A. Surfing the p53 network. *Nature* 2000; **408**: 307–310.
40 Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* 1993; **362**: 857–860.
41 Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997; **387**: 296–299.
42 Wu X, Bayle J, Olson D, Levine A. The p53-mdm-2 autoregulatory feedback loop. *Genes Dev* 1993; **7**: 1126–1132.
43 Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. *Nat Rev Cancer* 2009; **9**: 701–713.
44 Arai N, Nomura D, Yokota K, Wolf D, Brill E, Shohat O *et al.* Immunologically distinct p53 molecules generated by alternative splicing. *Mol Cell Biol* 1986; **6**: 3232–3239.
45 Matlashewski G, Pim D, Banks L, Crawford L. Alternative splicing of human p53 transcripts. *Oncogene Res* 1987; **1**: 77–85.
46 Flaman JM, Waridel F, Estreicher A, Vannier A, Limacher JM, Gilbert D *et al.* The human tumour suppressor gene p53 is alternatively spliced in normal cells. *Oncogene* 1996; **12**: 813–818.
47 Courtois S, Verhaegh G, North S, Luciani M-G, Lassus P, Hibner U *et al.* DeltaN-p53, a natural isoform of p53 lacking the first transactivation domain, counteracts growth suppression by wild-type p53. *Oncogene* 2002; **21**: 6722–6728.
48 Bourdon J-C, Fernandes K, Murray-Zmijewski F, Liu G, Diot A, Xirodimas DP *et al.* p53 isoforms can regulate p53 transcriptional activity. *Genes Dev* 2005; **19**: 2122–2137.
49 Ray PS, Grover R, Saumitra, Das S. Two internal ribosome entry sites mediate the translation of p53 isoforms. *EMBO Rep* 2006; **7**: 404–410.
50 Candeias MM, Powell DJ, Roubalova E, Apcher S, Bourougaa K, Vojtesek B *et al.* Expression of p53 and p53/47 are controlled by alternative mechanisms of messenger RNA translation initiation. *Oncogene* 2006; **25**: 6936–6947.

51 Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L, Benchimol S. Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene. *EMBO J* 1984; **3**: 3257–3262.
52 Fujita K, Mondal A, Horikawa I, Nguyen G, Kumamoto K, Sohn J *et al.* p53 isoforms |[Delta]|133p53 and p53|[beta]| are endogenous regulators of replicative cellular senescence. *Nat Cell Biol* 2009; **11**: 1135–1142.
53 Hofstetter G, Berger A, Fiegl H, Slade N, Zorić A, Holzer B *et al.* Alternative splicing of p53 and p73: the novel p53 splice variant p53δ is an independent prognostic marker in ovarian cancer. *Oncogene* 2010; **29**: 1997–2004.
54 Murray-Zmijewski F, Lane D. Bourdon J-C. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. *Cell Death Differ* 2006; **13**: 962–972.
55 Marcel V, Vijayakumar V, Fernández-Cuesta L, Hafsi H, Sagne C, Hautefeuille A *et al.* p53 regulates the transcription of its Δ133p53 isoform through specific response elements contained within the TP53 P2 internal promoter. *Oncogene* 2010; **29**: 2691–2700.
56 Chen J, Ng S, Chang C, Zhang Z, Bourdon J, Lane D *et al.* p53 isoform 113p53 is a p53 target gene that antagonizes p53 apoptotic activity via BclxL activation in zebrafish. *Genes Dev* 2009; **23**: 278–290.
57 Brady CA, Jiang D, Mello SS, Johnson TM, Jarvis LA, Kozak MM *et al.* Distinct p53 transcriptional programs dictate acute DNA-damage responses and tumor suppression. *Cell* 2011; **145**: 571–583.
58 Ghosh A, Stewart D, Matlashewski G. Regulation of human p53 activity and cell localization by alternative splicing. *Mol Cell Biol* 2004; **24**: 7987–7997.
59 Hafsi H, Lyon F, Santos-Silva D, Courtois-Cox S, Hainaut P. Ecully 69130. Effects of Δ40p53, an isoform of p53 lacking the N-terminus, on transactivation capacity of the tumor suppressor protein p53. *BMC Cancer* 2013; **13**: 134.
60 Holmila R, Fouquet C, Cadranel J, Zalcman G, Soussi T. Splice mutations in the p53 gene: case report and review of the literature. *Hum Mutat* 2003; **21**: 101–102.
61 Bourdon J-C, Khoury MP, Diot A, Baker L, Fernandes K, Aoubala M *et al.* p53 mutant breast cancer patients expressing p53γ have as good a prognosis as wild-type p53 breast cancer patients. *Breast Cancer Res* 2011; **13**: R7.
62 Avery-Kiejda K, Zhang X, Adams L, Scott R, Vojtesek B, Lane D *et al.* Small molecular weight variants of p53 are expressed in human melanoma cells and are induced by the DNA-damaging agent cisplatin. *Clin Cancer Res* 2008; **14**: 1659–1668.
63 Marabese M, Marchini S, Marrazzo E, Mariani P, Cattaneo D, Fossati R *et al.* Expression levels of p53 and p73 isoforms in stage I and stage III ovarian cancer. *Eur J Cancer* 2008; **44**: 131–141.
64 Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S *et al.* A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994; **266**: 66–71.
65 Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J Clin Oncol* 2007; **25**: 1329–1333.
66 Wu L, Wang Z, Tsan J, Spillman M, Phung A, Xu X *et al.* Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nat Genet* 1996; **14**: 430–440.
67 The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff [Internet]. (Accessed on 10 June 2013), available from http://www.hgmd.org/.
68 Karppinen S-M, Heikkinen K, Rapakko K, Winqvist R. Mutation screening of the BARD1 gene: evidence for involvement of the Cys557Ser allele in hereditary susceptibility to breast cancer. *J Med Genet* 2004; **41**: e114.
69 Ghimenti C, Sensi E, Presciuttini S, Brunetti I, Conte P, Bevilacqua G *et al.* Germline mutations of the BRCA1-associated ring domain (BARD1) gene in breast and breast/ovarian families negative for BRCA1 and BRCA2 alterations. *Genes Chromosom Cancer* 2002; **33**: 235–242.
70 Thai TH, du F, Tsan JT, Jin Y, Phung A, Spillman MA *et al.* Mutations in the BRCA1-associated RING domain (BARD1) gene in primary breast, ovarian and uterine cancers. *Hum Mol Genet* 1998; **7**: 195–202.
71 Irminger-Finger I, Soriano JV, Vaudan G, Montesano R, Sappino AP. In vitro repression of Brca1-associated RING domain gene, Bard1, induces phenotypic changes in mammary epithelial cells. *J Cell Biol* 1998; **143**: 1329–1339.
72 Irminger-Finger I, Leung W-C, Li J, Dubois-Dauphin M, Harb J, Feki A *et al.* Identification of BARD1 as mediator between proapoptotic stress and p53-dependent apoptosis. *Mol Cell* 2001; **8**: 1255–1266.
73 McCarthy E, Celebi J, Baer R, Ludwig T. Loss of Bard1, the heterodimeric partner of the brca1 tumor suppressor, results in early embryonic lethality and chromosomal instability. *Mol Cell Biol* 2003; **23**: 5056–5063.
74 Irminger-Finger I, Jefford C. Is there more to BARD1 than BRCA1? *Nat Rev Cancer* 2006; **6**: 382–391.
75 Capasso M, Devoto M, Hou C, Asgharzadeh S, Glessner JT, Attiyeh EF *et al.* Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. *Nat Genet* 2009; **41**: 718–723.
76 Wang K, Diskin S, Zhang H, Attiyeh E, Winter C, Hou C *et al.* Integrative genomics identifies LMO1 as a neuroblastoma oncogene. *Nature* 2010; **469**: 216–220.
77 Capasso M, Diskin S, Totaro F, Longo L, Mariano M, Russo R *et al.* Replication of GWAS-identified neuroblastoma risk loci strengthens the role of BARD1 and affirms the cumulative effect of genetic variations on disease susceptibility. *Carcinogenesis* 2013; **34**: 605–611.
78 Feki A, Jefford C, Berardi P, Wu J-Y, Cartier L, Krause K-H *et al.* BARD1 induces apoptosis by catalysing phosphorylation of p53 by DNA-damage response kinase. *Oncogene* 2005; **24**: 3726–3736.
79 Tsuzuki M, Wu W, Nishikawa H, Hayami R, Oyake D, Yabuki Y *et al.* A truncated splice variant of human BARD1 that lacks the RING finger and ankyrin repeats. *Cancer Lett* 2006; **233**: 108–116.
80 Wu J-Y, Vlastos A-T, Pelte M-F, Caligo M-A, Bianco A, Krause K-H *et al.* Aberrant expression of BARD1 in breast and ovarian cancers with poor prognosis. *Int J Cancer* 2006; **118**: 1215–1226.
81 Scully R, Chen J, Ochs R, Keegan K, Hoekstra M, Feunteun J *et al.* Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell* 1997; **90**: 425–435.
82 Jefford C, Feki A, Harb J, Krause K-H, Irminger-Finger I. Nuclear--cytoplasmic translocation of BARD1 is linked to its apoptotic activity. *Oncogene* 2004; **23**: 3509–3520.
83 Li L, Ryser S, Dizin E, Pils D, Krainer M, Jefford CE *et al.* Oncogenic BARD1 isoforms expressed in gynecological cancers. *Cancer Res* 2007; **67**: 11876–11885.
84 Ryser S, Dizin E, Jefford C, Delaval B, Gagos S, Christodoulidou A *et al.* Distinct roles of BARD1 isoforms in mitosis: full-length BARD1 mediates Aurora B degradation, cancer-associated BARD1 scaffolds Aurora B and BRCA2. *Cancer Res* 2009; **69**: 1125–1134.
85 Dizin E, Irminger-Finger I. Negative feedback loop of BRCA1--BARD1 ubiquitin ligase on estrogen receptor alpha stability and activity antagonized by cancer-associated isoform of BARD1. *Int J Biochem Cell Biol* 2010; **42**: 693–700.
86 Sporn J, Hothorn T, Jung B. BARD1 expression predicts outcome in colon cancer. *Clin Cancer Res* 2011; **17**: 5451–5462.
87 Zhang Y-Q, Pilyugin M, Kuester D, Leoni VP, Li L, Casula G *et al.* Expression of oncogenic BARD1 isoforms affects colon cancer progression and correlates with clinical outcome. *Br J Cancer* 2012; **107**: 675–683.
88 Zhang Y-Q, Bianco A, Malkinson AM, Leoni VP, Frau G, De Rosa N *et al.* BARD1: an independent predictor of survival in non-small cell lung cancer. *Int J Cancer* 2012; **131**: 83–94.
89 Bosse K, Diskin S, Cole K, Wood A, Schnepp R, Norris G *et al.* Common variation at BARD1 results in the expression of an oncogenic isoform that influences neuroblastoma susceptibility and oncogenicity. *Cancer Res* 2012; **72**: 2068–2078.
90 Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* 1988; **240**: 889–895.
91 Taplin M-E. Drug Insight: role of the androgen receptor in the development and progression of prostate cancer. *Nat Clin Pract Oncol* 2007; **4**: 236–244.
92 Knudsen KE, Scher HI. Starving the addiction: new opportunities for durable suppression of AR signaling in prostate cancer. *Clin Cancer Res* 2009; **15**: 4792–4798.
93 Shang Y, Myers M, Brown M. Formation of the androgen receptor transcription complex. *Mol Cell* 2002; **9**: 601–610.
94 Lubahn DB, Brown TR, Simental JA, Higgs HN, Migeon CJ, Wilson EM *et al.* Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. *Proc Natl Acad Sci USA* 1989; **86**: 9534–9538.
95 Shaffer PL, Jivan A, Dollins DE, Claessens F, Gewirth DT. Structural basis of androgen receptor binding to selective androgen response elements. *Proc Natl Acad Sci USA* 2004; **101**: 4758–4763.
96 Matias PM, Donner P, Coelho R, Thomaz M, Peixoto C, Macedo S *et al.* Structural evidence for ligand specificity in the binding domain of the human androgen receptor. Implications for pathogenic gene mutations. *J Biol Chem* 2000; **275**: 26164–26171.
97 Ahrens-Fath I, Politz O, Geserick C, Haendler B. Androgen receptor function is modulated by the tissue-specific AR45 variant. *FEBS J* 2005; **272**: 74–84.
98 Ikonen T, Palvimo J, Jänne O. Heterodimerization is mainly responsible for the dominant negative activity of amino-terminally truncated rat androgen receptor forms. *FEBS Lett* 1998; **430**: 393–396.
99 Jenster G, Korput H, Vroonhoven C, Kwast T, Trapman J, Brinkmann A *et al.* Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. *Mol Endocrinol* 1991; **5**: 1396–1404.
100 Huggins C, Hodges CV. Studies on prostatic cancer. *Cancer Res* 1941; **1**: 293–297.
101 Attard G, Reid A, Olmos D, Bono J. Antitumor activity with CYP17 blockade indicates that castration-resistant prostate cancer frequently remains hormone driven. *Cancer Res* 2009; **69**: 4937–4940.
102 Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V *et al.* Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* 2009; **324**: 787–790.

103 Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg C *et al.* In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nat Genet* 1995; **9**: 401–406.
104 Brinkmann AO. Molecular basis of androgen insensitivity. *Mol Cell Endocrinol* 2001; **179**: 105–109.
105 Ris-Stalpers C, Verleun-Mooijman MC, de Blaeij TJ, Degenhart HJ, Trapman J, Brinkmann AO. Differential splicing of human androgen receptor pre-mRNA in X-linked Reifenstein syndrome, because of a deletion involving a putative branch site. *Am J Hum Genet* 1994; **54**: 609–617.
106 Lim J, Ghadessy FJ, Yong EL. A novel splice site mutation in the androgen receptor gene results in exon skipping and a non-functional truncated protein. *Mol Cell Endocrinol* 1997; **131**: 205–210.
107 Hellwinkel OJ-C, Bull K, Holterhus P-M, Homburg N, Struve D, Hiort O. Complete androgen insensitivity caused by a splice donor site mutation in intron 2 of the human androgen receptor gene resulting in an exon 2-lacking transcript with premature stop-codon and reduced expression. *J Steroid Biochem Mol Biol* 1999; **68**: 1–9.
108 Hellwinkel OJ, Holterhus PM, Struve D, Marschke C, Homburg N, Hiort O. A unique exonic splicing mutation in the human androgen receptor gene indicates a physiologic relevance of regular androgen receptor transcript variants. *J Clin Endocrinol Metab* 2001; **86**: 2569–2575.
109 Brüggenwirth HT, Boehmer AL, Ramnarain S, Verleun-Mooijman MC, Satijn DP, Trapman J *et al.* Molecular analysis of the androgen-receptor gene in a family with receptor-positive partial androgen insensitivity: an unusual type of intronic mutation. *Am J Hum Genet* 1997; **61**: 1067–1077.
110 Tepper CG, Boucher DL, Ryan PE, Ma A-H, Xia L, Lee L-F *et al.* Characterization of a novel androgen receptor mutation in a relapsed CWR22 prostate cancer xenograft and cell line. *Cancer Res* 2002; **62**: 6606–6614.
111 Libertini S, Tepper C, Rodriguez V, Asmuth D, Kung H, Mudryj M. Evidence for calpain-mediated androgen receptor cleavage as a mechanism for androgen independence. *Cancer Res* 2007; **67**: 9001–9005.
112 Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res* 2008; **68**: 5469–5477.
113 Marcias G, Erdmann E, Lapouge GG, Siebert C, Barthélémy P, Duclos B *et al.* Identification of novel truncated androgen receptor (AR) mutants including unreported pre-mRNA splicing variants in the 22Rv1 hormone-refractory prostate cancer (PCa) cell line. *Hum Mutat* 2010; **31**: 74–80.
114 Hu R, Dunn T, Wei S, Isharwal S, Veltri R, Humphreys E *et al.* Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009; **69**: 16–22.
115 Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen HH *et al.* A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res* 2009; **69**: 2305–2313.
116 Jagla M, Feve M, Kessler P, Lapouge G, Erdmann E, Serra S *et al.* A splicing variant of the androgen receptor detected in a metastatic prostate cancer exhibits exclusively cytoplasmic actions. *Endocrinology* 2007; **148**: 4334–4343.
117 Sun S, Sprenger CCT, Vessella RL, Haugk K, Soriano K, Mostaghel EA *et al.* Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *J Clin Invest* 2010; **120**: 2715–2730.
118 Hu R, Isaacs WB, Luo J. A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. *Prostate* 2011; **71**: 1656–1667.
119 Zhang X, Morrissey C, Sun S, Ketchandji M, Nelson PS, True LD *et al.* Androgen receptor variants occur frequently in castration resistant prostate cancer metastases. *PLoS One* 2011; **6**: e27970.
120 Watson PA, Chen YF, Balbas MD, Wongvipat J, Socci ND, Viale A *et al.* Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. *Proc Natl Acad Sci USA* 2010; **107**: 16759–16765.
121 Chan SC, Li Y, Dehm SM. Androgen receptor splice variants activate androgen receptor target genes and support aberrant prostate cancer cell growth independent of canonical androgen receptor nuclear localization signal. *J Biol Chem* 2012; **287**: 19736–19749.
122 Cocquerelle C, Daubersies P, Majérus MA, Kerckaert JP, Bailleul B. Splicing with inverted order of exons occurs proximal to large introns. *EMBO J* 1992; **11**: 1095–1098.
123 Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. Preiss T (ed) *PLoS One* 2012; **7**: e30733.
124 Dreyfuss G, Matunis M, Pinol-Roma S, Burd C. hnRNP Proteins and the biogenesis of mRNA. *Annu Rev Biochem* 1993; **62**: 289–321.
125 Manley J, Tacke R. SR proteins and splicing control. *Genes Dev* 1996; **10**: 1569–1579.
126 Wu JY, Maniatis T. Specific interactions between proteins implicated in splice site selection and regulated alternative splicing. *Cell* 1993; **75**: 1061–1070.
127 Graveley BR, Hertel KJ, Maniatis T. The role of U2AF35 and U2AF65 in enhancer-dependent splicing. *RNA* 2001; **7**: 806–818.
128 Matlin AJ, Clark F, Smith CWJ, Clark C. Understanding alternative splicing: towards a cellular code. *Nat Rev Mol Cell Biol* 2005; **6**: 386–398.
129 Mayeda A, Helfman DM, Krainer AR. Modulation of exon skipping and inclusion by heterogeneous nuclear ribonucleoprotein A1 and pre-mRNA splicing factor SF2/ASF. *Mol Cell Biol* 1993; **13**: 2993–3001.
130 Crawford JB, Patton JG. Activation of alpha-tropomyosin exon 2 is regulated by the SR protein 9G8 and heterogeneous nuclear ribonucleoproteins H and F. *Mol Cell Biol* 2006; **26**: 8791–8802.
131 Li X, Manley J. Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability. *Cell* 2005; **122**: 365–378.
132 Karni R, Stanchina E, Lowe S, Sinha R, Mu D, Krainer A *et al.* The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nat Struct Mol Biol* 2007; **14**: 185–193.
133 Karni R, Hippo Y, Lowe S, Krainer A. *The splicing-factor oncoprotein SF2/ASF activates mTORC1. Proc Natl Acad Sci USA 2008;* ***105****: 15323–15327.*
134 Tang Y, Horikawa I, Ajiro M, Robles AI, Fujita K, Mondal AM *et al.* Downregulation of splicing factor SRSF3 induces p53β, an alternatively spliced isoform of p53 that promotes cellular senescence. *Oncogene* 2013; **32**: 2792–2798.
135 David C, Chen M, Assanah M, Canoll P, Manley J. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature* 2009; **463**: 364–368.
136 Babic I, Anderson ES, Tanaka K, Guo D, Masui K, Li B *et al.* EGFR mutation-induced alternative splicing of max contributes to growth of glycolytic tumors in brain cancer. *Cell Metab* 2013; **17**: 1000–1008.
137 Boukakis G, Patrinou-Georgoula M, Lekarakou M, Valavanis C *et al.* Biotechnology IHospital P. Deregulated expression of hnRNP A/B proteins in human non-small cell lung cancer: parallel assessment of protein and mRNA levels in paired tumour/non-tumour tissues. *BMC Cancer* 2010; **10**: 434.
138 Ghigna C, Moroni M, Porta C, Riva S, Biamonti G. Altered expression of heterogeneous nuclear ribonucleoproteins and SR factors in human colon adenocarcinomas. *Cancer Res* 1998; **58**: 5818–5824.
139 Piekielko-Witkowska A, Wiszomirska H, Wojcicka A, Poplawski P, Boguslawska J, Tanski Z *et al.* Disturbed expression of splicing factors in renal cancer affects alternative splicing of apoptosis regulators, oncogenes, and tumor suppressors. *PLoS One* 2010; **5**: e13690.
140 Lefave CV, Squatrito M, Vorlova S, Rocco GL, Brennan CW, Holland EC *et al.* Splicing factor hnRNPH drives an oncogenic splicing switch in gliomas. *EMBO J* 2011; **30**: 4084–4097.
141 Ponta H, Sherman L, Herrlich P. CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 2003; **4**: 33–45.
142 Cheng C, Sharp P. Regulation of CD44 alternative splicing by SRm160 and its potential role in tumor cell invasion. *Mol Cell Biol* 2005; **26**: 362–370.
143 Cappellari M, Bielli P, Paronetto M, Ciccosanti F, Fimia G, Saarikettu J *et al.* The transcriptional co-activator SND1 is a novel regulator of alternative splicing in prostate cancer cells. *Oncogene* 2013 1–9.
144 Guo X, Branch P, Chen Q-R, Song Y, Wei J *et al.* SAIC-Frederick. Exon array analysis reveals neuroblastoma tumors have distinct alternative splicing patterns according to stage and MYCN amplification status. *BMC Med Genomics* 2011; **4**: 35.
145 Thorsen K, Sorensen K, Brems-Eskildsen A, Modin C, Gaustadnes M, Hein A *et al.* Alternative splicing in colon, bladder, and prostate cancer identified by exon array analysis. *Mol Cell Proteomics* 2008; **7**: 1214–1224.
146 Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R *et al.* Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* 2011; **478**: 64–69.
147 Druker B, Tamura S, Buchdunger E, Ohno S, Segal G, Fanning S *et al.* Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr--Abl positive cells. *Nat Med* 1996; **2**: 561–566.
148 Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA *et al.* Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 2010; **363**: 809–819.
149 Wilson T, Fridly J, Yan Y, Penuel E, Burton L, Chan E *et al.* Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. *Nature* 2012; **487**: 505–509.
150 Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian Z, Du J *et al.* Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature* 2012; **487**: 500–504.
151 Nazarian R, Shi H, Wang Q, Kong X, Koya R, Lee H *et al.* Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 2010; **468**: 973–977.
152 Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G *et al.* RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* 2011; **480**: 387–390.

153 Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L *et al.* Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995; **80**: 155–165.
154 Lorson C, Rindt H, Shababi M. Spinal muscular atrophy: mechanisms and therapeutic strategies. *Hum Mol Genet* 2010; **19**: R111–R118.
155 Hua Y, Sahashi K, Rigo F, Hung G, Horev G, Bennett C *et al.* Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. *Nature* 2011; **478**: 123–126.
156 Miller-Wideman M, Makkar N, Tran M, Isaac B, Biest N, Stonard R. Herboxidiene, a new herbicidal substance from Streptomyces chromofuscus A7847. Taxonomy, fermentation, isolation, physico-chemical and biological properties. *J Antibiot (Tokyo)* 1992; **45**: 914–921.
157 Nakajima H, Sato B, Fujita T, Takase S, Terano H, Okuhara M. New antitumor substances, FR901463, FR901464 and FR901465. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. *J Antibiot (Tokyo)* 1996; **49**: 1196–1203.
158 Sakai T, Sameshima T, Matsufuji M, Kawamura N, Dobashi K, Mizui Y. Pladienolides, new substances from culture of Streptomyces platensis Mer-11107. I. Taxonomy, fermentation, isolation and screening. *J Antibiot (Tokyo)* 2004; **57**: 173–179.
159 Bonnal S, Vigevani L, Valc| J, Vigevani J. The spliceosome as a target of novel antitumour drugs. *Nat Rev Drug Discov* 2012; **11**: 847–859.
160 Webb TR, Joyner AS, Potter PM. The development and application of small molecule modulators of SF3b as therapeutic agents for cancer. *Drug Discov Today* 2013; **18**: 43–49.
161 Kotake Y, Sagane K, Owa T, Mimori-Kiyosue Y, Shimizu H, Uesugi M *et al.* Splicing factor SF3b as a target of the antitumor natural product pladienolide. *Nat Chem Biol* 2007; **3**: 570–575.
162 Kaida D, Motoyoshi H, Tashiro E, Nojima T, Hagiwara M, Ishigami K *et al.* Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. *Nat Chem Biol* 2007; **3**: 576–583.
163 Fan L, Lagisetti C, Edwards CC, Webb TR, Potter PM. Sudemycins, novel small molecule analogues of FR901464, induce alternative gene splicing. *ACS Chem Biol* 2011; **6**: 582–589.
164 Folco E, Coil K, Reed R. The anti-tumor drug E7107 reveals an essential role for SF3b in remodeling U2 snRNP to expose the branch point-binding region. *Genes Dev* 2011; **25**: 440–444.
165 Furumai R, Uchida K, Komi Y, Yoneyama M, Ishigami K, Watanabe H *et al.* Spliceostatin A blocks angiogenesis by inhibiting global gene expression including VEGF. *Cancer Sci* 2010; **101**: 2483–2489.
166 Corrionero A, Minana B, Valcarcel J. Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A. *Genes Dev* 2011; **25**: 445–459.
167 Zhou Z, Licklider L, Gygi S, Reed R. Comprehensive proteomic analysis of the human spliceosome. *Nature* 2002; **419**: 182–185.
168 Liu HX, Zhang M, Krainer AR. Identification of functional exonic splicing enhancer motifs recognized by individual SR proteins. *Genes Dev* 1998; **12**: 1998–2012.
169 Liu HX, Chew SL, Cartegni L, Zhang MQ, Krainer AR. Exonic splicing enhancer motif recognized by human SC35 under splicing conditions. *Mol Cell Biol* 2000; **20**: 1063–1071.
170 Coulter LR, Landree MA, Cooper TA. Identification of a new class of exonic splicing enhancers by in vivo selection. *Mol Cell Biol* 1997; **17**: 2143–2150.
171 Fairbrother WG, Yeh R-F, Sharp PA, Burge CB. Predictive identification of exonic splicing enhancers in human genes. *Science* 2002; **297**: 1007–1013.
172 Fairbrother W, Yeo G, Yeh R, Goldstein P, Mawson M, Sharp P *et al.* RESCUE-ESE identifies candidate exonic splicing enhancers in vertebrate exons. *Nucleic Acids Res* 2004; **32**(Web Server): W187–W190.
173 Yeo G, Hoon S, Venkatesh B, Burge CB. Variation in sequence and organization of splicing regulatory elements in vertebrate genes. *Proc Natl Acad Sci USA* 2004; **101**: 15700–15705.
174 Yeo GW, Nostrand EL, Van, Liang TY, Jolla C, Van Nostrand EL. Discovery and analysis of evolutionarily conserved intronic splicing regulatory elements. *PLoS Genet* 2007; **3**: e85.
175 Wang Y, Ma M, Xiao X, Wang Z. Intronic splicing enhancers, cognate splicing factors and context-dependent regulation rules. *Nat Struct Mol Biol* 2012; **19**: 1044–1052.
176 Hong X, Scofield DG, Lynch M. Intron size, abundance, and distribution within untranslated regions of genes. *Mol Biol Evol* 2006; **23**: 2392–2404.
177 Marcel V, Perrier S, Aoubala M, Ageorges S, Groves M, Diot A *et al.* Δ160p53 is a novel N-terminal p53 isoform encoded by Δ133p53 transcript. *FEBS Lett* 2010; **584**: 4463–4468.