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Review

# Impact of alternative splicing on mechanisms of resistance to anticancer drugs

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ABSTRACT

A shared characteristic of many tumors is the lack of response to anticancer drugs. Multiple mechanisms of pharmacoresistance (MPRs) are involved in permitting cancer cells to overcome the effect of these agents. Pharmacoresistance can be primary (intrinsic) or secondary (acquired), i.e., triggered or enhanced in response to the treatment. Moreover, MPRs usually result in the lack of sensitivity to several agents, which accounts for diverse multidrug-resistant (MDR) phenotypes. MPRs are based on the dynamic expression of more than one hundred genes, constituting the so-called resistome. Alternative splicing (AS) during pre-mRNA maturation results in changes affecting proteins involved in the resistome. The resulting splicing variants (SVs) reduce the efficacy of anticancer drugs by lowering the intracellular levels of active agents, altering molecular targets, enhancing both DNA repair ability and defensive mechanism of tumors, inducing changes in the balance between pro-survival and pro-apoptosis signals, modifying interactions with the tumor microenvironment, and favoring malignant phenotypic transitions. Reasons accounting for cancer-associated aberrant splicing include mutations that create or disrupt splicing sites or splicing enhancers or silencers, abnormal expression of splicing factors, and impaired signaling pathways affecting the activity of the splicing machinery. Here we have reviewed the impact of AS on MPR in cancer cells.

## 1. Introduction

In humans, at least 20,000 genes encode a highly interindividual diverse proteome comprising between 250,000 and 1,000,000 proteins [1]. The comparison of these numbers suggests that the synthesis of such a great variety of proteins present in our body primarily requires the diverse expression of our genes, whose activity is regulated at the transcriptional, post-transcriptional, and translational levels [2]. One of the processes responsible for this proteome complexity is the variability in editing immature mRNA (pre-mRNA) by splicing. This process is regulated by a high number of "splicing factors". These are proteins involved in the cut of introns from strings of pre-RNA and the

*Abbreviations:* 3′SS, 3′ acceptor site; 5-FU, 5-fluorouracil; ABC, ATP-binding cassette; AML, acute myeloid leukemia; AR, androgen receptor; AS, alternative splicing; BC, breast cancer; BPS, branch point site; CAC, colon adenocarcinoma; CCA, cholangiocarcinoma; CES, carboxylesterase; CML, chronic myeloid leukemia; CSC, cancer stem cells; EMT, epithelia-mesenchymal transition; ENT1, equilibrative nucleoside transporter 1; ER, estrogen receptor; ESE, exonic splicing enhancers; ESS, exonic splicing silencers; EVs, extracellular vesicles; GC, gastric cancer; GB, glioblastoma; GIC, gastrointestinal cancer; GM, glioma; HCC, hepatocellular carcinoma; hnRNP, heterogeneous nuclear ribonucleoprotein; HR, homologous recombination; ISE, intronic splicing enhancers; ISS, intronic splicing silencers; KC, kidney cancer; LC, lung cancer; LK, leukemia; ML, melanoma; MPRs, mechanisms of pharmacoresistance; MRP, Multidrug resistance-associated protein; OCT1, organic cation transporter 1; OC, ovarian cancer; OS, overall survival; OSCC, oral squamous cell carcinoma; PARPi, PARP inhibitors; PC, prostate cancer; PDAC, pancreatic ductal adenocarcinoma; PKM, pyruvate kinase gene; PPT, polypyrimidine tract; SNP, single nucleotide polymorphism; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; SV, spliced variant; TKIs, tyrosine kinase inhibitors.

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subsequent paste of adjacent exons. Splicing factors are either regulatory proteins or direct components of a multi-megadalton ribonucleoprotein complex named the spliceosome, which acts at the same time as transcription occurs, by removal of introns and re-connection of exons as an integrated element of the gene regulatory network [3]. In "constitutive splicing", the process occurs at canonical sites of pre-mRNA, whereas in "alternative splicing" (AS), the cut-and-paste mechanism involves different splicing sites, which result in diverse forms of partial or complete removal or retention of introns and exons, respectively [4]. As splicing of a single pre-mRNA can produce more than one mature mRNA transcript called splicing variant (SV), multiple protein isoforms, which may be structurally and functionally dissimilar, could be generated by AS. Because more than 95% of human genes can undergo AS [4,5], this diversity substantially increases the coding capacity of our genome [6]. On the other hand, aberrant splicing has been associated with several diseases, including cancer.

Despite the marked heterogeneity among types of cancer, among tumors of the same type, or even among tumors of the same patient, a shared characteristic of many tumors is their lack of response to pharmacological treatment, which is accounted for by multiple mechanisms of pharmacoresistance (MPRs). This characteristic of cancer cells can be primary (intrinsic pharmacoresistance) or secondary (acquired pharmacoresistance) triggered or enhanced in response to the treatment, which altogether can lead to diverse multidrug-resistant (MDR) phenotypes. MPRs are based on the dynamically changing expression of more than one hundred genes, constituting the so-called resistome, which is markedly affected by AS [7]. MPRs have been classified into seven groups (Fig. 1) [8,9] depending on whether they hinder the efficacy of anticancer drugs by: i) lowering the intracellular amount of total drug either by reducing drug uptake (MPR-1a) or enhancing drug export (MPR-1b); ii) changing the proportion (MPR-2) of active agents inside cancer cells; iii) altering molecular targets (MPR-3); iv) enhancing both DNA repair ability and defensive mechanism of tumors (MPR-4); v) inducing changes in the balance between pro-survival and pro-apoptosis signals (MPR-5); vi) modifying interactions with the tumor microenvironment (MPR-6); and vii) favoring malignant phenotypic transitions (MPR-7). In the present review, we have analyzed the role of AS in determining the resistome of cancer cells in general. Nevertheless, it should be kept in mind that a precise understanding of the lack of response to pharmacotherapy in any specific type of cancer during clinical practice requires taking into account additional characteristics of the individual cancer phenotype, not considered here, that markedly affect the overall impact of MPRs on each patient outcomes.

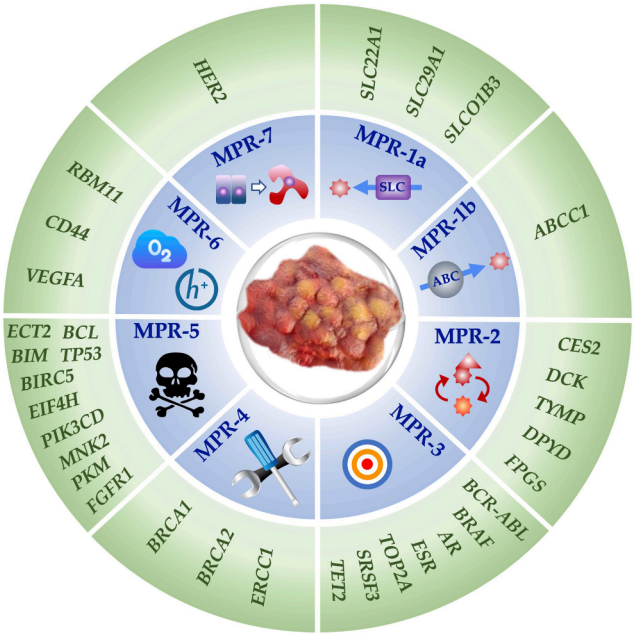


**Fig. 1.** Scheme of the seven groups of mechanisms of pharmacoresistance (MPR) and the genes of the resistome that are affected by alternative splicing in cancer cells.

## 2. The exon-recognition process

There are four types of introns. Those included in Groups I and II are removed through an autocatalytic reaction. Group I introns are found in some ribosomal RNA (rRNA), whereas Group II introns are found in rRNA, tRNA, and mRNA of fungal organelles, plants, and protists [10–13]. Group IV introns are found in tRNA genes. They need endonucleases and ligases to be processed [14]. Introns considered in the present review belong to Group III. These introns are removed by the spliceosome after the definition of their limits in pre-mRNA, which is based on their sequence and structure. In the exon-recognition process, a critical role is played by specific sequences: two located at the intron–exon junction, i.e., the 5′ donor site (5′SS) and the 3′ acceptor site (3′SS), and an internal sequence, the branch point site (BPS), located 18–40 nt from the 3′ ending edge of the intron. Group III introns can be classified into two subtypes, U2 and U12, depending on the class of spliceosome involved in their excision. Splicing of U2 introns, the most common type of introns in mammalian cells (more than 99% in humans) is performed by the so-called "major spliceosome" while the "minor spliceosome" is responsible for the removal of U12 introns [15–17].

The spliceosome is a multi-protein complex whose composition and conformation dynamically changes as exon-definition progresses from Complex E to subsequent Complexes A, B, and C (Fig. 2), before the cut-and-paste reactions start. The formation of the early E' complex triggers the beginning of the exon-recognition process. The nucleotide sequences of the pre-mRNA identified by the major spliceosome are GU at 5′SS and AG at 3′SS [18], whereas in the minor spliceosome, the equivalent sequences are AU and AC, respectively [17,19,20]. Major spliceosome assembly begins with a base pairing of GU at 5′SS with U1 small nuclear ribonucleoprotein (snRNP) (Fig. 2A). U1 snRNP belongs to a group of snRNPs consisting of: i) one of the five types (U1, U2, U4, U5, and U6) of small nuclear RNA (snRNA), which are RNA sequences of usually about 150 nucleotides in length; and ii) seven Sm proteins to generate the central region of the snRNP particle (Table 1). Sm proteins are necessary for the biogenesis, transport, and function of snRNP particles [21] and are crucial for their metabolic stability. In the case of U1 snRNP, the complex also includes three specific proteins, namely, U1 snRNP 70 kDa, U1A snRNP, and U1C snRNP [20] (Table 1). Moreover, during the exon-definition process, the splicing factor 1 (Sf1) binds to the BPS in an ATP-independent manner (Fig. 2A). The early E complex is then formed by recruitment of the U2 heterodimer (U2af), consisting of two subunits of 35 kDa (U2af1) and 65 kDa (U2af2), which bind to the 3′SS and the polypyrimidine tract (PPT), respectively [22] (Fig. 2B, Table 1).

The next step is the formation of the A complex, in which snRNP-U2 displaces Sf1, in an ATP-dependent manner. This process is catalyzed by the RNA helicases DDX46 (PRP5 in yeasts) and DDX39B (SUB2 in yeasts). DDX46/PRP5 assists in the binding of U2-subunit with U1-subunit [23], whereas DDX39B/SUB2 is required to stabilize the interaction between U2 subunit and pre-mRNA BPS [24] (Fig. 2C, Table 1). To generate the next structure, U4/U6-U5 tri-snRNP recruitment to the donor site occurs, forming the pre-catalytic B complex (B1), U5 binds to the 5′ end of the exon, and U6 binds to U2 (Fig. 2D).

In the next step, U1 and U4 snRNPs are released, which results in the activation of the spliceosome (activated B complex or B2 complex) [25]. The triggered catalytic activity of pre-mRNA processing factors included in the B2 complex [26], mediates the first reaction of splicing, producing an intermediate with lariat structure containing the 3′ intron loop [27] (Fig. 2E). Next, the spliceosome undergoes a conformational change, becoming the C complex, in which a different catalytically active appears [28,29]. Thus, U6 and U2 catalyze the second step, which by

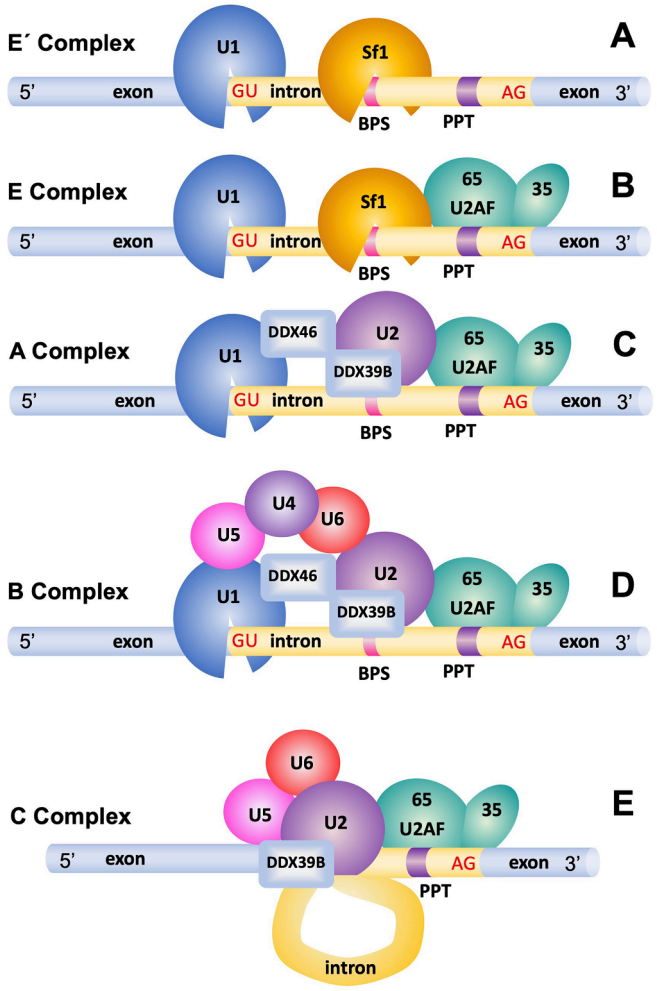


**Fig. 2.** Simplified scheme of exon-recognition process during splicing. (A) The splicing factor 1 (Sf1) binds to the BPS. (B) The early E complex is formed by recruitment of the U2 heterodimer U2af1/U2af2, which bind to the 3′SS and the polypyrimidine tract (PPT). (C) To generate the A complex, snRNP-U2 displaces Sf1, which is catalyzed by the RNA helicases DDX46 and DDX39B. (D) The recruitment of U4/U6-U5 tri-snRNP to the donor site occurs, forms the pre-catalytic B1 complex. (E) U1 and U4 snRNPs are released, resulting in the activation of the spliceosome (B2 complex), which is followed by the first reaction of splicing, producing an intermediate with lariat structure containing the 3′ intron loop, forming the C complex.

transesterification reactions, will join the exons after removing the intron with the loop structure, which is rapidly degraded by the cell [30–32]. In the minor spliceosome, ribonucleoproteins involved in this process are U11, U12, U4ATAC, U5, and U6ATAC [18].

A critical point in the splicing process is that the spliceosome must identify specific regions at the intron–exon borders to perform intron excision and exon binding accurately, despite the complexity accounted by the fact that exons constitute less than 1.1% of the human genome versus the 25% of introns. Differences in guanine and cytosine content between exons and introns, together with the existence of the aforementioned tag sequences (5′SS, 3′SS, BPS and PPT), make it possible to identify the exons for their processing by the spliceosome. Besides, there are *cis*-regulatory sequences named according to their position and function as exonic splicing enhancers (ESE), exonic splicing silencers (ESS), intronic splicing enhancers (ISE), and intronic splicing silencers (ISS) (Fig. 3). The definition of sequences to be removed depends partly on the interaction of pre-mRNA with many regulatory proteins that interact with these sites [18,20,33,34]. Thus, SR proteins (Table 1), characterized by a domain rich in arginine and serine di-peptides, favor short splicing due to their ability to bind to ESE and ISE [35]. SR proteins are concentrated in interchromatin granules or speckles [36], where splicing occurs [37] (Fig. 3). In contrast, heterogeneous nuclear ribonucleoproteins (hnRNPs) (Table 1) favor long splicing by inhibiting short splicing through interaction with both ESS and ISS [38] (Fig. 3). Besides, several kinases and phosphatases (Table 1) are involved in regulating of the spliceosome by changing the activity of SR proteins through phosphorylation and dephosphorylation, respectively [39]. The degree of complexity due to AS of pre-mRNA in target genes is increased because components of the splicing machinery and regulatory proteins involved in exon-recognition can themselves undergo AS, generating different isoforms (Table 1), which can modify the overall result of the splicing process.

## 3. Genetic events affecting the spliceosome and regulatory proteins in cancer

Since splicing dysregulation is a hallmark of cancer, it is not surprising that there are more than 15,000 tumor-associated SVs described in a wide variety of malignancies [40,41]. For instance, in a cell model of breast cancer, 1723 splicing alterations in target genes and changes in the expression of 41 splicing factors have been reported [42]. In most cases, these variants result from aberrant splicing, which produces proteins that alter, often favoring, critical aspects of cancer cell biology [43–45]. Besides generating abnormal proteins, AS can produce aberrant forms of mRNAs containing "poison exons", which lead to enhanced sequestration and degradation of specific "healthy" mRNAs in the nucleus, and hence alter cancer cells phenotype [46].

The mechanisms accounting for alterations in splicing observed in cancer cells include: i) abnormal expression of spliceosome components and regulatory proteins, which may affect their own splicing and hence the functional balance among their isoforms (Table 1) [47]; ii) mutations in these proteins; iii) mutations that create or disrupt splicing donor or acceptor sites or splicing enhancers or silencers in target pre-mRNAs [48,49]; and iv) changes in cell signaling pathways affecting the activity of the splicing machinery [48,50] (Table 1). Moreover, non-coding RNAs (ncRNAs), which include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and small nuclear RNAs (snRNAs), have a marked impact on AS in cancer [51], which is yet not fully understood. Finally, nonsense-mediated mRNA decay (NMD) is a key mechanism for elimination of aberrant splicing isoforms. Accordingly, when NMD is impaired, aberrant transcripts persist in the tumor. UPF1 is the most commonly altered NMD factor in cancer. Besides downregulation, *UPF1* often harbors splice site mutations itself [52].

Essential components of the U2 complex are SF3 proteins (Table 1). One of them is SF3B1, which has been identified as a driver protein in many cancers [53]. Regarding core proteins involved in E complex formation, the expression of SNRPB and SNRPD1 is higher in several tumors as compared with adjacent tissue. This is the case of hepatocellular carcinoma (HCC), in which up-regulation of both splicing factors has been correlated with shorter overall survival (OS) [54]. The expression of SNRPB is also increased in gliomas [55,56] and in non-small cell lung cancer (NSCLC) [57]. In these cancers, the tumorigenic capacity of SNRPB seems to be mediated, in part, by an SV of RAB26 that includes intron 7, whose generation is promoted by up-regulated SNRPB [57]. Altered expression of several hnRNPs has been found to be associated with cancer development in several organs. Thus, hnRNPE1 (PCBP1) acts as a tumor suppressor and is down-regulated in many cancers. In contrast, hnRNPE2 (PCBP2) and hnRNPK are considered oncogenes and promote tumorigenesis [58–61]. Among SR proteins, SRSF1, SRSF3, SRSF5, and SRSF9, which are considered indirect oncogenes, are up-regulated in several tumors [62–66]. Furthermore, as mentioned above, pre-mRNA of splicing factors (e.g., SRSF3) can undergo AS resulting in altered proteins with impact on the overall function of the spliceosome leading to changes in resistome profile and

**Table 1**
Components of the splicing machinery and regulatory proteins involved in exon-recognition, existence of splicing variants (SV), cancer-associated changes and known impact on mechanisms of pharmacoresistance (MPR).

| Role | Gene (Protein) | SV | Change | MPR | Ref. |
|---|---|---|---|---|---|
| They form part of U1 snRNP. | *SNRNP70* (U1 snRNP 70kD) | 2 | nd | | |
| Essential in the recognition of 5′SS and subsequent spliceosome assembly. | *SNRPA* (U1A snRNP) | 1 | nd | | |
| | *SNRPC* (U1C snRNP) | 1 | nd | | |
| Sm proteins constitute the central region of the snRNP particles U1, U2, U4/U6, and U5, which are involved in Complex E, A, B, and C. | *SNRPB/B1* (sm-B/B1) | 2 | + | | [55,57] |
| | *SNRPD1* (sm-D1) | 2 | + | | [54] |
| | *SNRPE* (sm-E) | 4 | nd | | |
| | *SNRPF* (sm-F) | 2 | nd | | |
| | *SNRPG* (sm-G) | 6 | nd | | |
| | *SNRPN* (sm-N) | 3 | nd | | |
| Interaction with U1 and U2 snRNPs. | *SF1* (Sf1) | 11 | -/* | | [54] |
| Recognition of 3′SS and PPT. | *U2AF1* (U2af1) | 3 | -/* | | [148] |
| Initial assembly of Complex E. | *U2AF2* (U2af2) | 2 | * | | [149] |
| Part of U2 snRNP. | *SF3A1* (SF3A1) | 1 | nd | | |
| Critical role in spliceosome assembly. | *SF3A2* (SF3A2) | 1 | nd | | |
| | *SF3A3* (SF3A3) | 2 | nd | | |
| | *SF3B1* (SF3B1) | 3 | +/* | 5, 7 | [102,143,150–154]. |
| | *SF3B2* (SF3B2) | 3 | nd | | |
| | *SF3B3* (SF3B3) | 1 | + | | [130,155] |
| | *SF3B4* (SF3B4) | 1 | nd | | |
| Helicases interacting with U2 snRNP. | *DDX46* (DDX46) | 2 | nd | | |
| They are involved in Complex A, B and C | *DDX39B* (DDX39B) | 1 | nd | | |
| SR proteins can modify the selection of splicing sites. | *SRSF1* (SFE2/ASF) | 2 | +/- | 5, 7 | [62,66,102,105,143] |
| They bind to intronic (ISE) and exonic (ESE) splicing enhancer sites stimulating short splicing. | *SRSF2* (SC35) | 1 | -/* | | [156,157] |
| They contain an RNA recognition motif (RRM) for binding RNA and an RS domain rich in serine and arginine residues for binding other proteins. | *SRSF3* (SRp20) | 1 | +/- | 1 | [62–67] |
| SR proteins are also involved in mRNA export from the nucleus and in translation. | *SRSF4* (SRp75) | 1 | – | | |
| | *SRSF5* (SRp40) | 3 | +/- | | [62–66] |
| | *SRSF6* (SRp55) | 1 | nd | | |
| | *SRSF7* (9G8) | 3 | – | | [54] |
| | *SRSF8* (SRp46) | 1 | – | | [54] |
| | *SRSF9* (SRp30c) | 1 | + | | [62–66] |
| | *SRSF10* (TASR) | 1 | + | | [65] |
| | *SRSF11* (NET2) | 9 | nd | | |
| | *SRSF12* (SREK1) | 2 | nd | | |
| | *SRRM1* (SRm160) | 26 | – | | [54] |
| | *RBM5* (LUCA15) | 7 | nd | | |
| hnRNPs can modify the selection of splicing sites. | *HNRPA1* (hnRNPA1) | 2 | nd | 3, 5 | [94,95,122] |
| They bind to intronic (ISS) and exonic (ESS) splicing silencer sites, inhibiting short splicing. | *HNRNPAB* (hnRNP A/B) | 2 | + | 7 | [140] |
| Participates in the transport of mRNA with poly(A) from the nucleus to the cytoplasm. | *HNRNPA2B1* (hnRNP B1) | 3 | nd | | |
| | *HNRNPD* (hnRNP D) | 4 | +/- | | [54,158] |
| | *PCBP1 or HNRNPE1* (PCBP1) | 1 | – | 5 | [58,59,121] |
| | *PCBP2 or HNRNPE2* (PCBP2) | 7 | + | | [60,61] |
| | *HNRNPF* (hnRNP F) | 1 | nd | | |
| | *HNRNPH1* (hnRNP H1) | 4 | + | | [145] |
| | *HNRNPH2* (hnRNP H2) | 2 | nd | 2 | [85] |
| | *HNRNPI* (PTBP1) | 3 | + | 1 | [81] |
| | *HNRNPK* (hnRNP K) | 4 | + | | [159–161] |
| | *HNRNPL* (hnRNP L) | 2 | +/- | | [162,163] |
| | *HNRNPM* (hnRNP M) | 3 | + | 7 | [133] |
| CDC-like kinases (CLKs) are nuclear kinases involved in the phosphorylation of SR proteins in the nucleus. | *CLK1* (CLK1) | 2 | – | | [54] |
| | *CLK2* (CLK2) | 4 | nd | | |
| | *CLK3* (CLK3) | 2 | nd | | |
| | *CLK4* (CLK4) | 1 | nd | | |
| SR protein kinases (SRPKs) are cytoplasmic kinases that phosphorylate SR proteins. | *SRPK1* (SRPK1) | 1 | nd | 5 | [120] |
| They specifically phosphorylate their substrates in serine residues located in regions rich in arginine/serine dipeptides. | *SRPK2* (SRPK2) | 9 | nd | | |
| Serine/threonine specific phosphatases involved in the regulation of SR proteins by removing phosphate groups. | *PPP1CA* (PP1A) | 3 | nd | | |
| | *PPP2CA* (PP2A) | 2 | nd | | |

Reported changes in cancer: +, up-regulation; -, down-regulation; *, mutations; nd, not described.

subsequent enhancement of pharmacoresistance. Thus, SRSF3 pre-mRNA undergoes AS affecting exon 4. The short SV lacking exon 4 encodes the full-length SRSF3 protein. In contrast, the long isoform, which includes exon 4 (SRSF3ex4), encodes a truncated SRSF3 protein due to an earlier termination codon, leading to enhanced resistance to paclitaxel in breast cancer and oral squamous cell carcinoma (OSCC) cells [67].

## 4. Role of AS in MPR-1. Changes in drug transporters

Mechanisms affecting the response of cancer cells to pharmacotherapy can be modified by the expression of SVs (Fig. 1, Table 2). This is the case of MPR-1, which accounts for changes in the uptake and efflux of antitumor drugs in cancer cells. The SLC superfamily of proteins currently includes 458 transporters classified into 65 families that transport a wide variety of substances across cell membranes; some of them play a crucial role in drug uptake [68]. An example is the organic cation transporter 1 (OCT1, *SLC22A1*), which is expressed at the basolateral membrane of hepatocytes and other healthy cells. This transporter plays an important role in the uptake of some antitumor drugs such as imatinib and sorafenib by cancer cells [69]. Several OCT1 SVs have been identified. Most of them are generated by exon skipping, which results in shorter OCT1 isoforms without transport activity. Thus, non-functional SVs, mainly due to exon 10, exon 9, and exon 9 + 10 skipping, are frequently expressed in HCC and cholangiocarcinoma (CCA) [70]. Besides, some OCT1 SVs have been associated with the lack of response of chronic myeloid leukemia (CML) to imatinib [71] and HCC to sorafenib [72,73].

The equilibrative nucleoside transporter 1 (ENT1, *SLC29A1*) is involved in the uptake of some nucleoside-related drugs. An intronic mutation in ENT1 that disrupts the splicing of exon 13 causes impaired cytarabine uptake in leukemia cell lines [74].

Organic anion transporter polypeptide 1B3 (OATP1B3, *SLCO1B3*) is involved in the uptake of anticancer drugs, such as paclitaxel, docetaxel, methotrexate, SN-38, platinum-based drugs, and several tyrosine kinase inhibitors (TKIs). Under physiological conditions, the so-called liver-type isoform (Lt-OATP1B3) is highly expressed at the basolateral plasma membrane of hepatocytes. However, in several primary solid tumors generated in the colon, lung, pancreatic, ovarian, prostate, bladder, and breast, the cancer-type variant (Ct-OATP1B3) has been identified [75–77]. Compared with Lt-OATP1B3, the transcription start site of *Ct-OATP1B3* mRNA is delayed to the second intron of this gene, and hence, the predicted *Ct-OATP1B3* ORF lacks part of the N-terminal coding region of Lt-OATP1B3 [78,79]. Although it is still unclear, there is evidence supporting that Ct-OATP1B3 has minimal or negligible transport activity. Nevertheless, Ct-OATP1B3 seems to confer proliferation advantage and chemotherapy resistance to cancer cells [79,80].

Multidrug resistance-associated protein 1 (MRP1, *ABCC1*) is one of the ATP-binding cassette (ABC) pumps able to export anticancer drugs from cancer cells, conferring pharmacoresistance. More than twenty SVs of MRP1 have been described in cancer (e.g., ovarian cancer), with a higher expression in tumor than in adjacent non-tumor tissue. These SVs are generated by partial exon skipping and intron inclusion, mainly between exons 10 and 19. Interestingly, the paired analysis in the same tumor samples, revealed enhanced expression of splicing factors polypyrimidine tract binding protein 1 (PTBP1, *HNRNPI*) and SRp20 (*SRSF3*) (Table 1). The in vitro study of the functional repercussions of these MPR1 SVs showed that MRP1–2 k (exons 12–26 skipped and 99 nucleotides of exon 27 skipped), MRP1-d5d30 (exon 5 and 30 skipped), and MRP1-d17d18 (exon 17 and 18 skipped) still conferred doxorubicin resistance. However, they were not as potent as the full-length wild-type MRP1 [81].

## 5. Role of AS in MPR-2. Changes affecting pro-drug activation and drug inactivation

Mechanisms altering the proportion of active versus inactive drugs inside cancer cells are classified as MPR-2 (Fig. 1, Table 2). Carboxylesterases (CES) catalyze the hydrolysis of a wide variety of compounds. CES2 is involved in the intracellular activation of irinotecan into its active metabolite SN-38. Six SVs can be formed by combining different ATG start sites and two splicing events. Alternate 5′-splicing in exon 10 removes 48 nucleotides from *CES2* pre-mRNA generating the CES2Δ458-473 variant, which lacks 16 amino acids in the proximity of the active site resulting in impaired esterase activity. Consequently, the conversion of irinotecan into SN-38 is hampered. Nevertheless, since CES2Δ458-473 constitutes only a small proportion (6%) of total CES2, at least in colon cancer, it is unclear that this AS could play an essential role in determining irinotecan pharmacokinetics and therapeutic outcome of these patients [82].

Deoxycytidine kinase (*DCK*) is an intracellular enzyme required to activate cytarabine. Several SVs have been identified in cytarabine-resistant acute myeloid leukemia (AML), which are absent from sensitive leukemia cells and healthy leukocytes [83,84]. Besides, SVs lacking exon 2 and exon 3, due to intronic mutations, were found in cytarabine-resistant leukemia cells [74].

Thymidine phosphorylase (TP, *TYMP*) is a crucial enzyme in the first step of metabolic biotransformation of 5-fluorouracil (5-FU) into the active metabolite fluorodeoxyuridine monophosphate. Leukemia cells with enhanced 5-FU resistance present partly un-spliced *TYMP* pre-mRNA, which results in introns 1–6 still included in the major transcript. The consequence is the presence of a premature stop codon in this SV which is translated into a dysfunctional enzyme unable to activate 5-FU. The generation of this SV has been associated with an enhanced nuclear abundance of hnRNPH2, which acts as an inhibitor of exon recognition [85].

Dihydropyrimidine dehydrogenase (*DPD*) is the main enzyme accounting for capecitabine catabolism. *DPD* overexpression induces a lack of sensitivity to capecitabine. However, on the other hand, reduced DPD activity may result in enhanced drug toxicity in patients receiving capecitabine. The rs3918290 SNP located in intron 14 is associated with an alteration of the splicing donor site, which results in the generation of the variant DPYD*2A by exon 14 skipping. Besides, the rs67376798 SNP located in exon 22 probably generates a broken ESE site together with a new ESS site. These changes alter exon recognition, which produces aberrant transcripts that are translated into proteins with deficient enzymatic activity. These variants have been significantly associated with increased capecitabine-induced toxicity in patients treated with this drug [86].

The intracellular retention of folates and antifolates drugs, such as methotrexate depends on conjugation through their gamma-carboxyl residue with multiple glutamate moieties, which is catalyzed by the enzyme folyl-poly-gamma-glutamate synthetase (FPGS), whose activity is reduced in acute lymphoblastic leukemia cells with decreased sensitivity to these drugs. This can be due in part to aberrant splicing, which results in a dysfunctional SVs generated by exon 12 skipping and intron 8 partial retention [87–89].

## 6. Role of AS in MPR-3. Changes affecting drug targets

The group of MPR-3 includes changes affecting drug targets (Fig. 1, Table 2). Imatinib is a TKI targeting the oncogenic BCR-ABL fusion protein, used to treat some malignancies, such as chronic myeloid leukemia (CML). In this cancer, point mutations affecting the kinase domain of the *BCR-ABL* gene often result in imatinib resistance. Besides, SVs, such as BCR-ABL35INS, which leads to premature termination of the protein and results in an inactive kinase domain, have been associated with inadequate response to imatinib treatment [83].

Vemurafenib inhibits cell growth acting on the RAS-MAPK pathway.

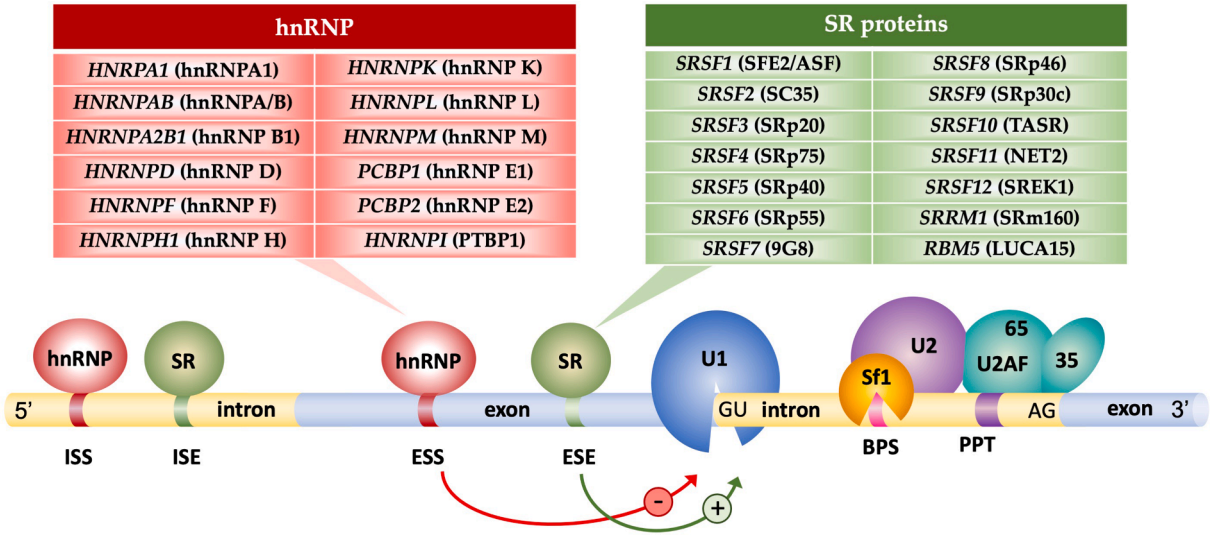


**Fig. 3.** Role of major regulatory proteins (SRs and hnRNPs) in exon definition. BPS, branch point site; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; ISE, intronic splicing enhancer; ISS, intronic splicing silencer; PPT, polypyrimidine tract.

This antitumor drug is used in melanoma treatment because it inhibits the BRAF p.V600E, which is the most frequent isoform in this skin cancer [90]. However, a shorter protein (C3 variant) encoded by a SV of BRAF p.V600E, generated by exon 4–8 skipping, is found in melanomas characterized by vemurafenib resistance [91,92]. The appearance of this SV leads to constitutive dimerization of BRAF p.V600E in a RAS-independent manner. Interestingly, using both in vitro and in vivo models, it has been shown the spliceostatin A (or its analog meayamycin B), a compound that targets SF3B1, can reverse the resistance to vemurafenib in melanomas harboring BRAF p.V600E SVs [93].

The androgen receptor (AR) is a critical therapeutic target for treating some tumors. The SV AR-V7, which has been detected in hormone-refractory prostate cancer, presents exons 4–7 skipping, which generates a functional protein lacking the ligand-binding domain. Thus, tumors expressing AR-V7 exhibit resistance to anti-AR drugs, such as enzalutamide and abiraterone. In vitro studies have demonstrated that hnRNPA1 inhibition by quercetin can prevent the generation of AR-V7 and re-sensitize prostate cancer cells to enzalutamide [94,95].

Tamoxifen is used in the treatment of estrogen receptor (ER)-positive breast cancer. However, in some tumors, SV ERα36 is expressed. In this SV, the transcription is initiated from different promoters located in the first intron. Hence, ERα36 lacks the transcriptional activation domains and shows a worse tamoxifen response than tumors expressing the full-length wild-type ERα [96].

Acquired resistance to TOP2α inhibitors, such as mitoxantrone, etoposide, teniposide, daunorubicin, and doxorubicin, is frequently associated with a decreased level of TOP2α expression. Similarly, several truncated isoforms of TOP2α originated by AS resulting in reduced drug activity against TOP2α [97].

Ten-eleven translocation-2 (TET2) modulates DNA hydroxymethylation by converting 5-methylcytosine into 5-hydroxymethylcytosine to promote DNA demethylation. Skipping of exon 2 in *TET2* pre-mRNA by AS results in cytarabine resistance of AML cells [7,98].

## 7. Role of AS in MPR-4. Changes affecting DNA repair

Some antitumor drugs, such as platinum-derived agents or inhibitors of poly(ADP-ribose) polymerase (PARP), induce double-stranded DNA breaks, which can be repaired by the homologous recombination (HR) DNA repair system. Enhanced tumor ability of DNA repair or protection have been classified as MPR-4 (Fig. 1, Table 2). BRCA1/2 proteins are essential for efficient HR-mediated DNA repair. Because tumors, such as breast or ovarian cancer, present a high frequency of loss-of-function mutations in *BRCA1* and *BRCA2* genes, these malignacies are potentially sensitive to PARP inhibitors (PARPi) and platinum-based treatment. However, in some cases, acquired resistance has been attributed to aberrant splicing of *BRCA1* pre-mRNA that generates the BRCA1-Δ11q variant, in which most part of exon 11, containing inactivating mutations of this gene, is skipped. Consequently, the sensitivity to PARPi and platinum therapy are partly lost [99]. In vitro assays revealed that the sensitivity to PARPi was restored using an inhibitor of the U2 snRNP spliceosome machinery [99]. Aberrant splicing of *BRCA2* has also been associated with acquired drug resistance. Thus, compared to the wild-type BCRA2 protein, the BRCA2ΔE5 + 7 SV, lacking exons 5 and 7, encodes an in-frame shorter protein due to the internal deletion of 55 amino acids. Hence, the expression of this SV induces mitomycin C resistance in AML cells [100].

An essential element of the nucleotide excision repair (NER) mechanism is excision repair cross complementation group-1 (ERCC1), capable of repairing cisplatin-induced DNA damage. In ovarian cancer, treatment with cisplatin favors the appearance of a functional SV of ERCC1, which results in a longer transcript and provides increased resistance to cisplatin [101].

## 8. Role of AS in MPR-5. Changes affecting apoptosis/survival balance

As most anticancer agents aim to activate apoptosis in the tumor selectively, the imbalance between pro-apoptotic and pro-survival factors commonly occurring in cancer cells hamper the response to pharmacotherapy, through processes classified as MPR-5 (Fig. 1, Table 2). Epithelial cell transforming 2 (ECT2) is a guanine nucleotide exchange factor involved in cell cycle regulation. ECT2-exon 5 inclusion levels are associated with poor prognosis in breast cancer after chemotherapy. This SV is favored by splicing factors ZRANB2 and SYF2 up-regulation, which leads to acquired resistance to doxorubicin [42].

Owing to aberrant splicing, the generation of SVs of members of the BCL-2 family promotes anticancer drug resistance. MCL-1, BCL-X, and BID have SVs coding for isoforms with opposite pro-apoptotic and anti-apoptotic activities. MCL-1 splicing is partially regulated by SF3B1, which explains why, in NSCLC and cervical carcinoma, SF3B1 inhibition reverses the predominance of SVs (differing in exon 2) from the anti-apoptotic MCL-1L (long) to pro-apoptotic MCL-1S (short) [102,103]. BCL-X can also undergo AS to produce either the long anti-apoptotic

**Table 2**
Splicing variants in genes associated with mechanisms of pharmacoresistance.

| MPR | Gene (Protein) | Splicing Event | Impact | Cancer | Ref. |
|---|---|---|---|---|---|
| 1 | *SLC22A1* (hOCT1) | TGGTAAGT insertion at exon 7 (rs113569197) | No functional transporter<br>Worse imatinib response | CML | [71] |
| | *SLC29A1* (ENT1) | Exon 13 skipping | Absence of plasma membrane expression of ENT1. Impaired cytarabine uptake in vitro | LK | [74] |
| | *SLCO1B3* (OATP1B3) | Ct-OATP1B3 (transcription start site in intron 2) | Minimal or negligible transport activity. Absence of plasma membrane location. Lack of transport ability so possible link with drug resistance | CAC, PDAC | [79,80] |
| | *ABCC1* (MRP1) | MRP1–2 k; MRP1-d5d30; MRP1-d17d18 (all resulting in exon skipping) | Maintained drug transport activity. Doxorubicine resistance in vitro, although less potent than the full-length MRP1 | OC | [81] |
| 2 | *CES2* (CES2) | $CES2\Delta^{458-473}$ | Impaired esterase activity. Impaired conversion of irinotecan into SN-38 in vitro | CAC | [82] |
| | *DCK (*DCK*)* | Exon (2–6) skipping | Inactive proteins. Linked with cytarabine resistance in AML cells and patients | AML | [74,83,84] |
| | *TYMP* (TP) | Introns (1– 6) retention | Lack of protein. 5-FU resistance in vitro | LK | [85] |
| | *DPYD* (DPD) | Intronic SNPs and aberrant splicing | Low functionality of the enzyme. Increase toxicity in breast cancer patients treated with capecitabine | BC | [86] |
| | *FPGS* (FPGS) | Exon 12 skipping and intron 8 partial retention | Impaired polyglutamylation leading to decreased intracellular drug retention<br>Lower response to methotrexate, dexamethasone, mitoxanthrone, and prednisolone | ALL | [9,87–89] |
| 3 | *BCR-ABL* (BCR-ABL) | BCR-ABL35INS (retention of 35 nt of intron 8) | Protein with an inactive kinase domain. Poor response to imatinib treatment | CML | [164] |
| | *BRAF* (BRAF) | BRAF C3 variant (exon 4–8 skipping) | Constitutive dimerization of BRAF. Resistance to vemurafenib in vitro and in vivo models. Vemurafenib resistance in melanoma patients | ML | [91–93] |
| | *AR* (AR) | AR-V7 (skipping of exons 4–7) | Protein lacking the ligand binding domain. Patients expressing AR-V7 exhibit resistance to enzalutamide and abiraterone | PC | [93] |
| | *ESR* (ER) | ERα36 (different promoter located in the first intron) | Protein lacking transcriptional activation domains. Worse patient response to tamoxifen | BC | [96] |
| | *TOP2A* (TOP2α) | Intron retention variants | Truncated protein.<br>Mitoxantrone resistance in vitro | AML | [97] |
| | *SRSF3* (SRSF3) | Exon inclusion (SRSF3ex4). Premature termination codon | Paclitaxel resistance | BC, OSCC | [67] |
| | *TET2* (TET2) | Exon 2 skipping | Impaired DNA demethylation. Cytarabine resistance | AML | [9,98] |
| 4 | *BRCA1* (BRCA1) | BRCA1-Δ11q (exon 11 skipping) | Bypass inactivating mutations of the protein. Partial resistance to PARPi and platinum therapy | BC, OC | [99] |
| | *BRCA2* (BRCA2) | $BRCA2\Delta^{E5+7}$ (exon 5 and 7 skipping) | Protein lacking 55 amino acids<br>Mitomycin C resistance in vitro | AML | [100] |
| | *ERCC1* (ERCC1) | Transcriptional start point located upstream of the normal one | Larger ERCC1 transcript. Cisplatin resistance | OC | [101] |
| 5 | *ECT2* (ECT2) | ZRANB2 and SYF2 up-regulation leading to *ECT2* exon 5 inclusion | Doxorubicin resistance | BC | [42] |
| | *BCL* (BCL) | BCL-XL (includes exon 2.1) | Antiapoptotic variant<br>Imatinib resistance in vitro and in vivo models | CML, AML | [104,165] |
| | *BIM* (BIM) | BIM-γ | Isoforms lacking the pro-apoptotic BCL2-homology domain 3 lead to imatinib resistance in vitro<br>Patients with this variant respond worst to imatinib | CML | [106] |
| | *TP53* (p53) | Δ133p53 (different promoter vs full length TP53) | Anti-p53 function. 5-FU resistance in vitro | CCA | [109] |
| | | Δ133p53 | Camptothecin resistance in vitro | CAC | [110] |
| | | Δ40p53 (initiation of translation within intron 2, and deletion of 40 amino acids) | Cisplatin resistance in vitro | ML | [111] |
| | | Alternative splicing of exon 9b or 9 g | Enhanced sensitivity to doxorubicin | AML | [112] |
| | *BIRC5* (Survivin) | Survivin-3B (intron 3 retention) | Inhibits procaspase-6<br>Cisplatin and 5-FU resistance in vitro | BC, CAC, KC | [113–115] |
| | | Survivin-2B (includes additional exon 2.B) | Blocks apoptosis induced by BAX Patients with this variant treated with cytarabine and mitoxantrone present worst OS | AML | [116] |
| | *EIF4H* (EIF4H) | RBM10-dependent aberrant splicing | Resistance to PI3K/AKT/mTOR inhibitors (e.g., AZD8055, BEZ235) | LC | [117] |
| | *PIK3CD* (PIK3CD) | Exon 20 skipping | Resistance to PI3K inhibitors, such as idelalisib and wortmannin | PC | [118] |
| | *MNK2* (MNK2) | Exclusion of part of the exon 13 | Gemcitabine resistance | PDAC | [9,119] |
| | *PKM* (Pyruvate kinase) | PKM2 (a mutually exclusive exon variant) | Gemcitabine resistance in vitro | PDAC | [121] |
| | *FGFR1* (FGFR1) | Inclusion of intronic segments. Premature termination codon | Enhanced pAKT activity<br>Resistance to FGFR1 inhibitors, such as dovitinib | SACC | [123] |
| 6 | *RBM11* (RBM11) | Enriched in EVs | Altered splicing profiles (of MDM4 and Cyclin D1. Impaired apoptosis activation. | GB | [125] |
| | *CD44* (CD44) | CD44v8-10 | Chemoresistance in vitro | BC, GIC, OC, PC | [136 137–139] |
| | *VEGFA* (VEGFA) | Alternative 3′ SS for exon 8 (VEGFA145b) | Resistance to bevacizumab | CAC | [129] |
| | Release of EVs containing SF3B3 and hnRNPA3 | | Drug resistance by altered apoptosis activation | AML | [126] |

(*continued on next page*)

**Table 2** (*continued*)

| MPR | Gene (Protein) | Splicing Event | Impact | Cancer | Ref. |
|---|---|---|---|---|---|
| | Splicing factors transported by tunnelling nanotubes | | Imatinib resistance in vitro | CML | [128] |
| 7 | *EZH2* (EZH2) | Exon 14 inclusion | Stem-cell-like epigenetic stateEnzalutamide resistance | PC | [130] |
| | *HER2* (HER2) | Δ16HER2, P100-HER2, and Herstatin | Sensitivity to trastuzumab | BC | [145] |

AML, acute myeloid leukemia; BC, breast cancer; CAC, colon adenocarcinoma; CCA, cholangiocarcinoma; CML, chronic myeloid leukemia; EVs, extracellular vesicles; GB, glioblastoma; GIC, gastrointestinal cancer; KC, kidney cancer; LC, lung cancer; LK, leukemia; ML, melanoma; OC, ovarian cancer; OSCC, of oral squamous cell carcinoma; PC, prostate cancer; PDAC, pancreatic ductal adenocarcinoma; SACC, salivary adenoid cystic carcinoma.

variant (BCL-XL) or the short pro-apoptotic one (BCL-XS). SF3B1 and SRSF1 partly orchestrate the generation of these SVs [102]. Thus, in cervical carcinoma, SRSF1 overexpression results in BCL-XL generation, whereas its down-regulation promotes BCL-XS formation [102]. In some cancers, the enhanced expression of BCL-XL often results in resistance to several drugs, such as imatinib [104]. The mechanism of action of this TKI involves stimulation of *BIM* (also named *BCL2L11*) transcription and post-translational activation of BIM protein, which induces apoptosis. AS of BIM may result in reduced sensitivity to imatinib and other TKIs. There is evidence indicating that SRSF1 promotes BIM AS [105]. Thus, the SV named BIM-γ, arising from exon 4-to-exon 3 switch due to an intronic deletion polymorphism, is correlated with resistance to imatinib and to other TKIs in CML and NSCLC cells [106,107].

AS of *TP53* pre-mRNA accounts for the generation of 12 SVs [108]. These include three smaller variants, named P53β, Δ40p53, and Δ133p53, which are associated with drug resistance. Thus, Δ133p53, which exhibits anti-p53 function, has been related to 5-FU resistance in CCA cells [109] and can protect colorectal cancer cells from camptothecin-induced apoptosis [110]. Besides, Δ40p53 expressed in melanoma cells inhibits p53-dependent transcription of p21 and PUMA and contributes to cisplatin resistance [111]. In contrast, AS can also generate proteins associated with a better prognosis. For instance, truncated forms of p53 produced by AS of exon 9β or 9γ correlates with a more satisfactory response of AML to doxorubicin due to the increased promoter binding ability of those isoforms, which up-regulates tumor suppressor (e.g., p21) and of pro-apoptotic (e.g., BAX) genes [112].

A survivin (*BIRC5*) SV named survivin-3B is originated by intron 3 retention. Survivin-3B binds and inhibits procaspase-6. Thus, this SV, which is highly expressed in cancer of several organs, such as breast, colon, and kidney, increases their resistance to cisplatin [113] and 5-FU [114,115]. Moreover, AS of *BIRC5* pre-mRNA can generate survivin-ΔEx2, lacking exon 2, and survivin-2B, which contains an additional exon 2B. In AML patients treated with cytarabine and mitoxantrone regime, those with leukemia cells expressing higher levels of survivin-2B showed poorer OS than those with lower expression [116].

*RBM10* encodes a nuclear protein that contains an RNA-binding motif through which RBM10 interacts with hnRNPs to regulate AS of its target genes, such as eukaryotic translation initiation factor 4H (EIF4H). Lung cancer patients with low RBM10 expression have reduced survival rates. RBM10-dependent aberrant splicing of EIF4H in cancer cells results in gain-of-function SVs leading to resistance to PI3K/AKT/mTOR inhibitors (e.g., AZD8055, BEZ235) [117].

Several mutations in PI3K result in constitutive activation of the PI3K/AKT/mTOR signaling pathway, which is associated with cancer progression. Two *PIK3CD* mRNAs are expressed in prostate cancer, i.e., *PIK3CD-L* (long variant) and *PIK3CD-S* (lacking exon 20). The latter is translated into a PI3Kδ short isoform that resists the inhibition induced by PI3K inhibitors, such as idelalisib and wortmannin, and has been associated with more aggressive tumors [118].

In pancreatic ductal adenocarcinoma (PDAC), MNK-dependent phosphorylation of eIF4E correlates with disease grade, early onset of disease, and worse prognosis. Gemcitabine up-regulates SRSF1, which favors the AS of MNK2 to generate MNK2b. This SV lacks part of the exon 13 and hence losses the binding site required to interact with MAPK, which enhances eIF4E phosphorylation and hence reduces gemcitabine-induced apoptosis and promotes cancer cell survival [7,119]. Moreover, experimentally induced expression of SRPK1 confers resistance to treatment with gemcitabine [120]. In contrast, SRSF1 silencing, using siRNA, abolishes MNK AS, reducing eIF4E phosphorylation and restoring the sensitivity of PDAC cells to gemcitabine [119].

In this sense, gemcitabine resistant-PDAC cells exhibited a switch in the AS of pyruvate kinase gene (*PKM*). This gene encodes two SVs, PKM1 and PKM2, through the incorporation of mutually exclusive exons. The latter is involved in tumor development. Moreover, PTBP1 is also up-regulated in pharmacoresistant PDAC cells, which promotes PKM2 generation. Besides, knockdown of PTBP1 precludes its binding to *PKM*

pre-mRNA, favoring the expression of PKM1 and restoring the sensitivity to gemcitabine [121]. Interestingly, miR-374b down-regulation has been shown to affect PKM1/PKM2 splicing by modifying hnRNPA1 expression in sorafenib resistant HCC cells [122].

Three novel, truncated FGFR1 variants (FGFR1v) have been identified in salivary adenoid cystic carcinoma (SACC) [123]. They are generated by premature termination codon by inclusion in the mature mRNA of intronic segments. These SVs are cell surface proteins lacking their intracellular domain, whose expression enhances pAKT activity, rendering SACC cells more resistant to FGFR1 inhibitors, such as dovitinib [7,123].

## 9. Role of AS in MPR-6. Changes affecting the tumor microenvironment

The surrounding microenvironment may also affect tumor cells altering their response to pharmacotherapy (MPR-6) (Fig. 1, Table 2). Splicing events have been detected both in the secretome [124] and in extracellular vesicles (EVs) derived from cancer cells [125], which may have an impact on the drug resistance of other cancer cells located in their vicinity. For example, EVs released by apoptosis-resistant AML cells contain an enriched amount of SF3B3 and hnRNPA3, which can be taken up by apoptosis-sensitive AML cells altering their drug resistance [126]. Another example is observed in glioblastoma (GB) cells, where apoptotic cells release EVs containing the splicing factor RBM11. This is a tissue-specific splicing regulator selectively expressed in the brain, cerebellum, and testis, and to a lower extent in the kidney [127]. Upon uptake of these EVs by non-apoptotic GB cells, RBM11 can alter the splicing profile of MDM4 and Cyclin D1 toward the expression of more oncogenic isoforms, thereby promoting their proliferation and drug resistance [125]. Tunneling nanotubes can mediate the transport of vesicles between bone marrow stromal cells and CML cells. The cargo of these vesicles, which is enriched in splicing factors, transfers the ability to protect CML cells from imatinib-induced apoptosis [128].

Angiogenesis is a key determinant of the tumor microenvironment. The use of an alternative 3′ SS for exon 8 definition in *VEGFA* pre-mRNA generates the SV VEGFA145b, which acts as a reservoir of angiogenic growth factors in the tumor stroma leading to bevacizumab resistance in colorectar adenocarcinoma [129].

## 10. Role of AS in MPR-7. Changes affecting phenotypic transition

In the group of MPR-7 changes favoring malignant phenotypic transitions such as EMT and the appearance of cancer stem cells (CSC)-like traits, have been included (Fig. 1, Table 2). EZH2, the catalytic subunit of the epigenetic modulator PRC2, promotes cancer development and progression through epigenetic silencing of tumor suppressors by trimethylation of histone H3. Exon 14 inclusion during splicing, which is promoted by SF3B3, results in an active SV accounting for stem-cell-like epigenetic state of prostate cancer cells that is accompanied by enzalutamide resistance [130]

Alterations in the expression of different splicing regulatory proteins have been described as precursors of EMT, which determines an unsatisfactory response to pharmacological treatment. In turn, during EMT, the expression of several components of the splicing machinery is markedly changed [131,132]. Thus, epithelial splicing regulatory proteins 1 (ESRP1) and 2 (ESRP2), are epithelial cell-specific regulators of AS. Both splicing factors are down-regulated during EMT. In breast cancer, the balance between ESRP1 and hnRNPM is critical for stimulating EMT and chemoresistance. Whereas hnRNPM favors mesenchymal phenotype due to its effect enhancing exon skipping and TGFβ signaling, ESRP1 favors epithelial phenotype by enhancing exon reading [133]. Both proteins act as competitive factors binding to *cis*-elements in target pre-mRNAs to antagonize theirs AS [134].

The CD44 gene, which is involved in EMT, has 10 constant exons and 10 variable exons (v1-v10) located in the pre-mRNA between constant exon 5 and 16. Thus, several SVs can be generated during AS by inclusion, which is promoted by ESRP1, or exclusion, favored by hnRNPs, of variable exons. CD44v6 and CD44v9 interact with CD95, the death receptor, interfering with death receptor signaling and inhibiting apoptosis, which results in chemoresistance [133,135]. Moreover, the expression of the variant containing exons v8-10 (CD44v8-10) has been associated with enhanced chemoresistance in gastrointestinal, breast, ovarian, and prostate cancer cells [136,137]. The up-regulation of this SV has been found in cisplatin-resistant urothelial cancer cells [138] and has been associated with docetaxel resistance in prostate cancer. Accordingly, the detection of CD44v8-10 mRNA in serum exosomes has been proposed as a prognostic marker [132,139].

The expression of *HNRNPAB* is increased in many cancers, including HCC, which favors EMT in a SNAIL-dependent manner [140]. Aberrant splicing in other genes such as *RON* and *RAC1B* also promotes EMT (MPR-7) and angiogenesis (MPR-6), which hamper the effect of anti-angiogenic drugs [141,142]. Moreover, the overexpression of the oncogenic SVs of the protein H-Ras in cancer cells results in the release of exosomes containing splicing factors such as SF3B1 and SRSF1, which are suggested to be taken up by distant cells where they promote EMT, leading to more tumor aggressiveness and poorer response to the treatment [143].

Three SVs of the transcription factor OCT4 have been described. In colon cancer cells, overexpression of the SV OCT4B1 promoted invasion and migration but also enhanced chemoresistance because OCT4B1 suppressed the sensitivity to oxaliplatin by up-regulating ABCB1 and ABCG2 export pumps [144].

In HER2-positive breast cancer, the presence of phenotypic changes toward that of CSCs, which correlates with enhanced aggressiveness and pharmacoresistance, has been associated with the appearance of aberrant SVs. This MPR-7 markedly depends on SRSF3 and hnRNPH1 expression. These changes are clinically relevant because the expression levels of these SVs (e.g., Δ16HER2, P100-HER2, and Herstatin) determine the response to trastuzumab [145].

## 11. Conclusions and Perspectives.

The information analyzed here permits to reach several valuable conclusions. In the first place, similarly to its impact on interindividual variability in healthy people, the existence of splice events increases the diversity of tumor cell phenotypes favoring tumor heterogeneity regarding crucial malignant characteristics, such as pharmacoresistance. Altered AS can result from changes in the expression and function of regulatory elements of the exon-recognition machinery in tumor cells, some of them affected by AS themselves, during carcinogenesis, progression, and response to treatment.

Considering the high frequency of aberrant splicing associated with cancer pathogenesis and therapy, pharmacological intervention to modulate AS may provide new therapeutic opportunities in cancer [146]. Several preclinical studies have investigated the usefulness of small molecules as modulators of splicing. An important drawback of this strategy is that the targets of most of these agents are constitutive components of the spliceosome, whose manipulation often results in adverse side effects. Nevertheless, certain selectivity for individual splice events has been identified, which opens a window of hope in this line of research [147]. This is important because cancer cells are markedly active regarding replication, which requires a high rate of pre-mRNA processing. Accordingly, it is reasonable to expect that cancer cells could be more sensitive than healthy quiescent cells to pharmacological manipulation of the splicing machinery using novel drugs.

## Author Contributions

M.R., M.S., E.L., M.A., O.M-A., F.S.M., and J.J.G.M have contributed substantially to the elaboration of the manuscript. All authors have read

and agreed to the published version of the manuscript.

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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.

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