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Dysregulation and Therapeutic Targeting of RNA Splicing Variants in Cancer

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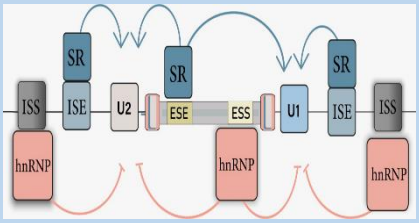
Abstract: mRNA splicing constitutes a crucial biological phenomenon characterized by the excision of introns from pre-mRNA, followed by the synthesis of mature mRNA through the concatenation of the remaining exons. In eukaryotic cells, the process of alternative splicing introduces a diverse array of exon combinations, thereby exertinga significant influence on the generation of protein diversity. The occurrence of recurrent mutations during RNA splicing or within the core components of the spliceosome is implicated in the etiology and progression of diverse diseases, prominently including cancer. This review article scrutinizes the aberrations in RNA splicing linked to cancer and explores therapeutic endeavors directed at addressing mutations in RNA splicing.



Keywords: Alternative splicing; spliseosome; splicing factors; splicing factor mutations; splice switch oligonucleotides

Introduction

RNA splicing is a biological post-transcriptional modification that happened in the nucleus of most eukaryotic species, where the newly synthesized pre-mRNA is processed to form the mature mRNA which then leaves to the cytoplasm of the cell and undergoes translation to synthesis a functional protein. While most eukaryotic genes are transcribed and processed, the newly formed mRNA from prokaryotic genes is completing the translation process without any further modifications after transcription.

In1970s, numerous studies described an alternative splicing, which is a molecular process where a single gene can be encoded to various mRNAs that translated to proteins with different structures and functions. In the human genome which harboring between 19,000 to 20,000 protein coding genes (1), up to 95% undergoes alternative RNA splicing to produce diverse protein isoforms (2-4).

Many studies have reported the association between aberrant alternative splicing events with many human diseases like cancer (5). In the present review, the process of splicing was explained. Next, the mechanism of alternative splicing and the dysregulations related to this process was discussed. And finally, we summarized the most recent strategies and technologies to target RNA splicing mutations especially in cancer.

Splicing Mechanism

The process of RNA splicing involves the excision of non-coding RNA sequences (introns), then joining the remaining coding RNA sequences (exons). This process is facilitated by the aid of spliceosome and regulated by different small nuclear ribonucleoproteins (snRNPs). The spliceosome, is a large complex that consists of 5 snRNAs and man other proteins. The assembly of U1,U2,U4,U6 and U5 snRNPs compromises the major spliceosome, while the assembly of U11,U12,U5,U6atac and U4atac snRNPs compromises the minor spliceosome (6). Introns exhibit four crucial sites: the 5' splice site (5'ss), the 3' splice site (3'ss) with GU- and AG- short dinucleotide sequences respectively, the branch point sequence (BPS), and the polypyrimidine tract (7) (Figure 1A).

Spliceosome assembly initiates with U1 snRNP binding to the 5'ss on pre-mRNA, followed by SF1 binding to the BPS near the 3'ss. Then U2 auxiliary factors; (U2AF1 and U2AF2) bind to the 3'ss and the upstream polypyrimidine tract, establishing an early complex (complex E) or prespliceosome (complex A). Substitution of SF1 with U2 snRNP, containing SF3B1, leads to prespliceosome formation, followed by association with preassembled tri-snRNPs U4/U5/U6, forming the pre-activated spliceosome (complex B). Conformational changes displace U1 and U4 snRNPs, forming the catalytically activated spliceosome (complex B*). Complex B* undergoes esterification reactions to produce catalytically active forms (complex C, C*). The cycle concludes with mature mRNA formation through the release of remaining splicing proteins, intron lariat, and exon ligation (8-10) (Figure 1B).

Alternatively, alternative splicing occurs when different exons are retained or excluded, generating alternative mRNA transcripts (11). The decision on exon inclusion or exclusion involves regulation by cis-regulatory elements (ESEs, ESSs, ISEs, ISSs) and trans-acting factors, including serine/arginine (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNP) family proteins (12-16) (Figure 2). Various RNA-binding proteins, such as NOVA (17), MBNL (18), CELF (19), and FOX (20), also regulate alternative splicing. Human cells exhibit several alternative splicing patterns, including exon skipping, alternative first exon, alternative last exon, intron retention, mutually exclusive exons, alternative 5' splicing sites, and alternative 3' splicing sites (21-23) (Figure 3).

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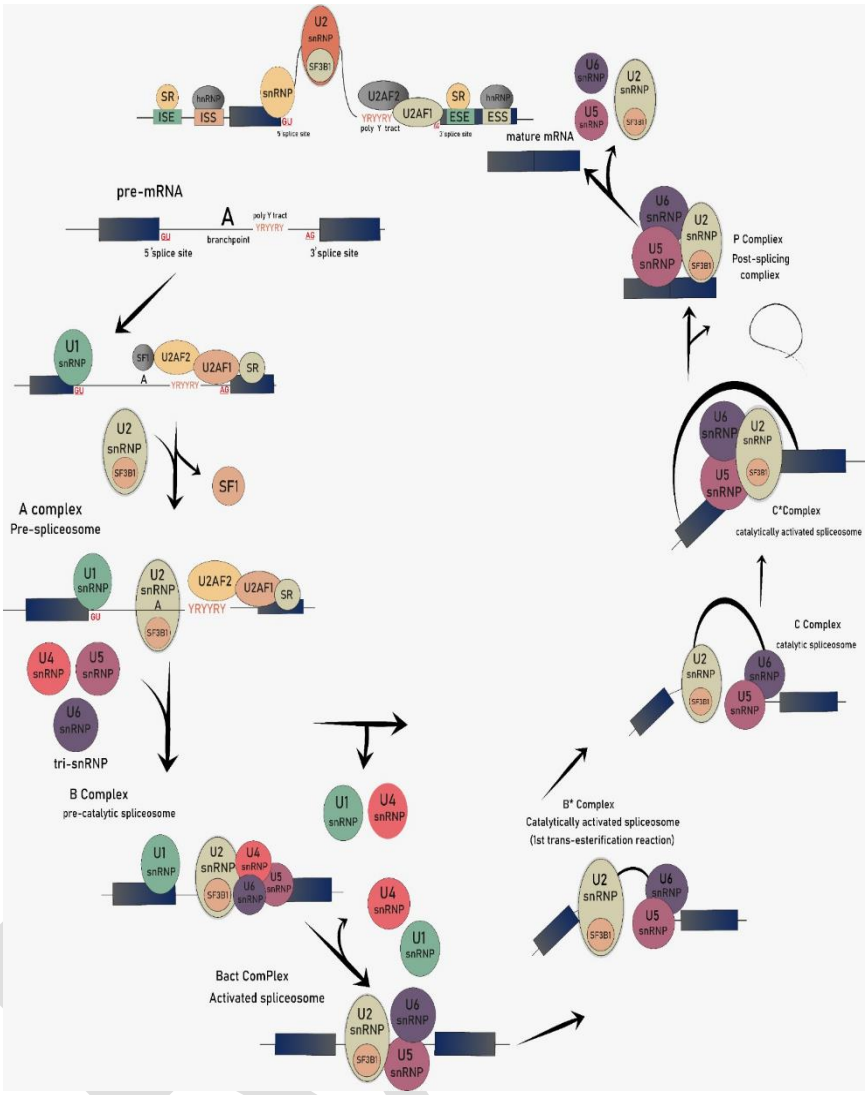

Figure (1): Schematic illustration of spliceosome assembly and the process of pre- mRNA splicing.

Crucially, splice variants originating from AS, possess the capability to produce distinct protein isoforms that may either lose or acquire specific domains, leading to variations in functionality. By using sophisticated technologies like (RNA-seq) and advances proteomics, numerous AS transcripts have been identified (24). It is of great importance to understand the mechanisms regulating splicing in both normal physiological body functions and disease conditions, which contributes in developing therapeutics to target splicing defects (25).

Recurrent Mutations Affecting RNA Splicing Factors in Cancer

Numerous investigations have supplied evidence establishing a connection between the dysregulation of alternative splicing and various diseases, notably cancer (26). The emergence and progression of cancer can be ascribed to mutations transpiring in vital components of the spliceosome or origin binding sequences of cis-acting splicing factors (27-29). Over 50% of patients diagnosed with myelodysplastic syndromes (MDS) exhibit spliceosome mutations, suggesting their potential involvement in disease development (30). Notably, mutations in splicing factors SF3B1, SRSF2, ZRSR2, and U2AF1 are frequently identified among MDS patients (31-33).

Unlike SF3B1, SRSF2, and U2AF1, which exhibit heterozygous missense mutations in hot-spot parts (Figure 4), ZRSR2 mutations are distributed throughout the gene and are hypothesized to induce impairment (34).

Mutations in SF3B1

SF3B1 splicing factor stands out as the most frequently mutated component across malignancies, particularly prevalent in cancer sub-types such as myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), uveal melanoma (UVM), acute myeloid leukemia (AML), and myeloproliferative neoplasm (MPN). Noteworthy is the distinct biomarker value of SF3B1 mutations for specific cancer forms (35-40). SF3B1, a component of the U2 snRNP in pre-spliceosome formation, binds to the branch point and recognizes the majority of 3'ss (41).

Splicing analysis, utilizing RNA sequence data from cancer cells harboring SF3B1 mutations, corroborates the finding that mutant cells with SF3B1 deviate from the canonical splicing pattern, employing an aberrant intron proximal 3'ss (42-45). In many tumor types, the poor prognosis is associated with SF3B1 mutations in hot spots, and consequently it cause global disruption of canonical splicing (46). In one of MDS types, ring sideroblasts in refractory anemia (RARS) enriched with SF3B1

mutations, exhibits an aberrant iron accumulation in the mitochondria forming a characteristic "ring" of blue granules.

Through structure - activity relationship studies, SF3B1 mutations identified to provide genetic vulnerability to a

The majority of SF3B1 mutations cluster near Heat Repeat domains (from 4 to 7) (HR4- HR7). Residues K700 and K666 are most commonly mutated in MDS and CLL, while the prevalent allele in uveal melanoma is an allele mutated at position R625. The functional implications of these unique mutations concerning disease sub-types remain uncertain, warranting further research (48, 49).

nongenotoxic molecule called UM4118 which act as copper ionophores in many acute myeloid leukemia (AML) patient samples. UM4118 initiates a mitochondrial based cell death (47).

A recent study reveals how cancer - associated SF3B1 mutations affect transcription; by using different cell lines, mouse model and patient samples. They discovered that the elongation rate of RNA polymerase II has reduced by SF3B1 mutations and its density near promoters have lowered. The distribution of pre-spliceosome assembly that caused by defective protein- protein interaction of mutant SF3B1 was the main cause of elongation defect (50).

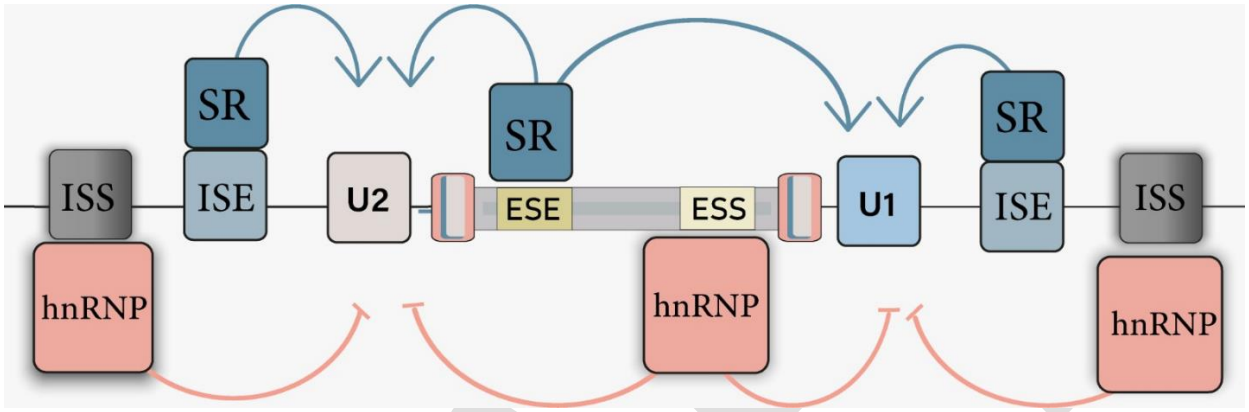

Figure (2): RNA splicing is orchestrated by cis-acting RNA sequences, including Intronic Splicing Enhancers (ISEs), Intronic Splicing Silencers (ISSs), Exonic Splicing Enhancers (ESEs), and Exonic Splicing Silencers (ESSs), which serve asbinding sites for RNA binding protein (RBP) splicing factors. Specifically, SR proteins are recruited to ESSs/ESEs, where they predominantly facilitate exon inclusion by interacting with U1 snRNP and U2 snRNP, which bind to the 5' splicesite and branch site, respectively. Concurrently, Heterogeneous Nuclear Ribonucleoproteins (hnRNPs) typically associate with ISSs/ISEs, engaging in competitive interactions with SR proteins and thereby exerting repression on splicesite selection.

Mutations in SRSF2

The impact of hot spot mutations in SRSF2 on splicing and disease development has been extensively studied. SRSF2, functioning as an auxiliary splicing factor, attenuates Exonic Splicing Enhancers (ESEs) to recruit the core spliceosome and facilitate splicing (51). SRSF2 is mutated in a minority of patients with disomy 3UM, chronic myelomonocytic leukemia (CMML), acute myeloid leukemia (AML), and high-risk myeloproliferative neoplasms (52).

SRSF2 typically promotes splicing by binding to exonic splicing enhancers (ESEs) abundant in C and G mutations in SRSF2 (53). These mutations, concentrating on residue P95, alter RNA-binding preference in favor of C-rich CCNGover G-rich ESEs, resulting in abnormal splicing of hundreds of mRNAs (54,55). A notable consequence in SRSF3-mutant cells is the disruption in the splicing of EZH2 mRNA, that encoding the histone methylation regulator, which accomplished by myeloid neoplasms's loss of function mutations. Nonsense-mediated decay targets the aberrant EZH2 mRNA which produced by the mutant SRSF2, and many mutations in EZH2 and SRSF2 are detected exclusively in MDS (56,57).

Mutations in U2AF1

U2AF1, along with its counterpart U2AF2, constitutes a heterodimeric U2AF complex facilitating the assembly of U2 snRNP complex to the Branch Point Site (BPS) (58). At the 3'ss, U2AF1 is associated with the AG dinucleotide, while U2AF2 is bound to the polyperimidine tract. Hotspot mutations in the U2AF1 predominantlyimpact residues Q157 or S34, situated in one of the protein's zinc fingers. These mutations are associated with specific cancer lineages, exemplified by U2AF1-S34 mutations prevalent in lung adenocarcinoma, while U2AF1-Q157 mutations are absent (59).

U2AF1-S34 mutations facilitate the molecular inclusion of exons with a C-rich 3'ss,whereas the U2AF1-Q157 mutations enhance the inclusion of exons with a G-rich 3'ss (60,61). Recurring hotspot mutations in PHF5A, a crucial U2 snRNP component interacting with SF3B1, have been recently identified in 119 patients across 33 types of solid tumors, adding to the list of splicing factors with hotspot mutations (62).

Mutations in ZRSR2

The somatic mutations of ZRSR2 are expected to disrupt the open reading frame and are dispersed throughout the coding region, often appearing as frame shift indels, nonsense mutations, or splice sites. X-linked ZRSR2 mutations are observed in many male patient samples of MDS and CMML (63). ZRSR2, a vital component of the minor spliceosome, facilitates the splicing of minor introns, constituting less than 1% of all introns in the human.

The absence of ZRSR2 results in increasing the retention of U12-type-containing introns, while the splicing of introns in U2-type remains essentially unaffected (52, 64, 65).

Approaches for Addressing Splicing Aberrations in many Cancer's types

According to an important role of dysregulated alternative splicing in the initiation and also the progression of cancer, substantial attention has been directed towards devising therapeutic strategies specifically targeting aberrant splicing in cancer. Diverse therapeutic approaches are presently advancing through distinct phases of clinical andpre-clinical

development. In this discourse, we discuss extant therapeutic methodologies that focus on the spliceosome, RNA binding proteins, splicing regulatory proteins, and oligonucleotide-based interventions.

Small molecule modulators against splicing in cancer

Numerous natural compounds, sourced from bacterial species, have been identified as binders to the SF3B complex. Notably, compounds such as spliceostatin A (66), E7107 (analogous to pladienolide B) (67), and sudemycins (68), impede the interaction between the branch point binding region containing U2 snRNP and the BPS, thereby hindering the crucial structural transition in U2 snRNP (69-72). Spliceostatin A and meayamicin B have demonstrated efficacy in rectifying splicing errors and overcoming vemurafenib resistance induced by p61 BRAF V600E through inhibition of exon skipping (73).

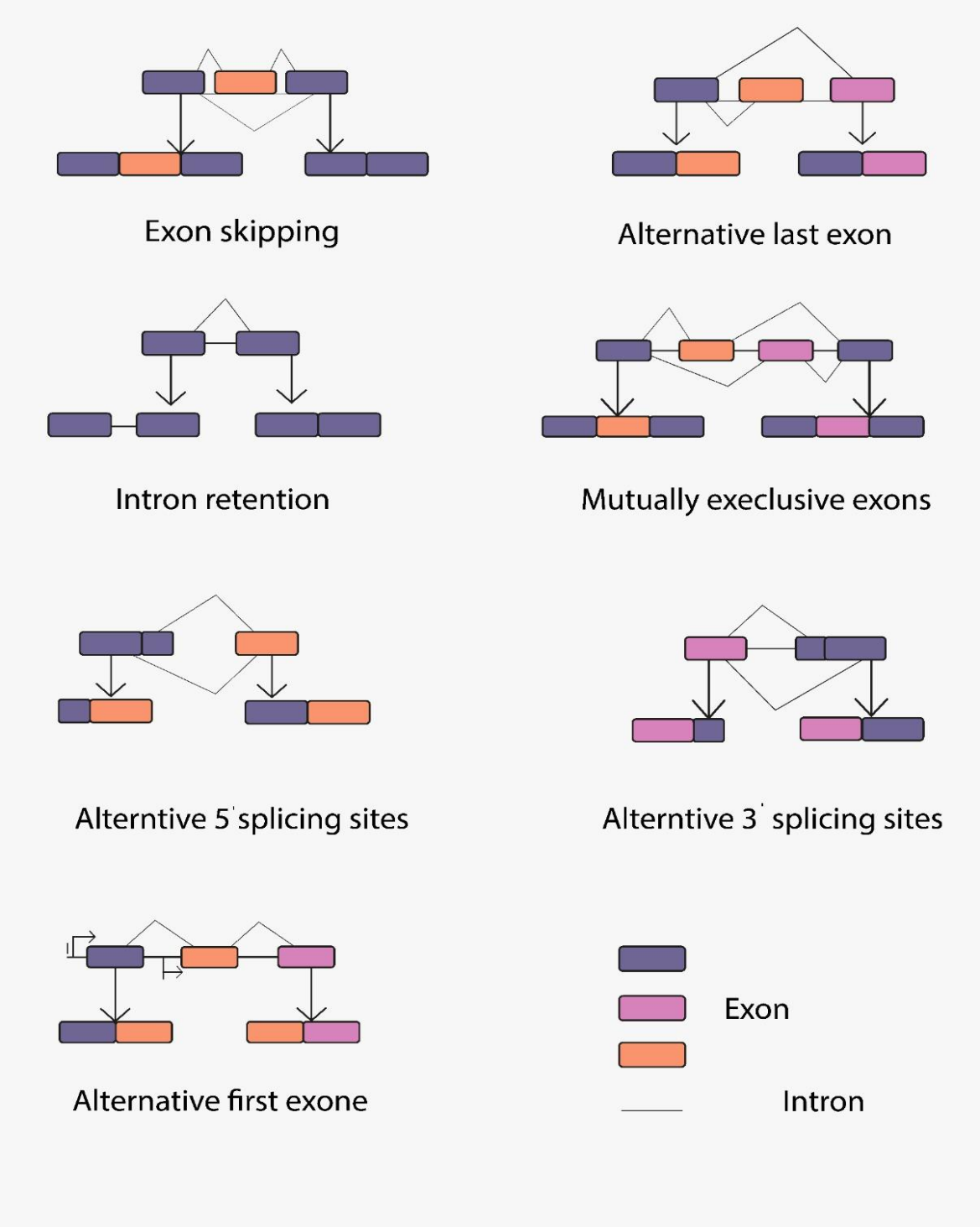

Figure (3). Various RNA splicing patterns encompass exon skipping, alternative initiation of transcription at the first exon, alternative termination of transcription at the last exon, retention of introns, selection between mutually exclusive exons, and utilization of alternative 5' and 3' splicing sites.

Other new small molecules that target various phases of the splicing pathway have been discovered, such as CDC-like kinases (CLKs), the SR protein kinases (SRPKs), and many PRMTs (74-76). The CLK family, collaborating with SRPKs, regulates the phosphorylation level of RS dipeptides on SR proteins, and alterations in CLK activity and expression have been linked to cancer progression. PRMT-5, a type II PRMT, symmetrically dimethylates arginine residues in splicing-regulating factor SmD3 (77-83) and is implicated in tumor development. Inhibiting PRMT-5 reduces cellular proliferation in cancer cell lines (84), making it a promising target in anticancer agent development (85-87).

Despite the limited effectiveness of sulfonamides as anticancer agents in cancer patients (88,89) , particularly in the absence of knowledge regarding prevalent SF mutations, recent studies have identified sulfonamides like indisulam and E7820 as molecular targets of RPM39 (90,91), an RPM crucial for splicing regulation. RPM39 depletion leads to global splicing alterations, which includes increased exon skipping and many intron retention, offering an alternative avenue for therapeutically targeting aberrant splicing in cancer.

Oligonucleotide-based therapy for alternative splicing

An additional strategy involves the development and application of antisense splice- switching oligonucleotides (ASOs) that form complementary base pairs with RNA. This category of treatments aims to either degrade RNA or influence splicing throughRNA hybridization. ASOs have been employed to modulate the splicing of NDM4, STAT3, and KRAS (92-95), effectively inhibiting tumor cell proliferation both in vitro and in vivo.

Alternative splicing directed by CRISPR-Cas9 in cancer treatment

The clustered regularly interspaced short palindromic repeats (CRISPR)- CRISPR Cas-9 associated protein is a component of ancient bacteria's adaptive immune system (96). To investigate the effect of SF3B1 mutations resulting in alternative splicing events in cancer cell lines, a novel CRISPR-Cas9 based system was employed (55). While further research is needed to fully understand how CRISPR-Cas9 targets oncogenic alternative splicing events, the system's ease of modification suggest that CRISPR-Cas9 is a useful tool for targeting splicing mutations .

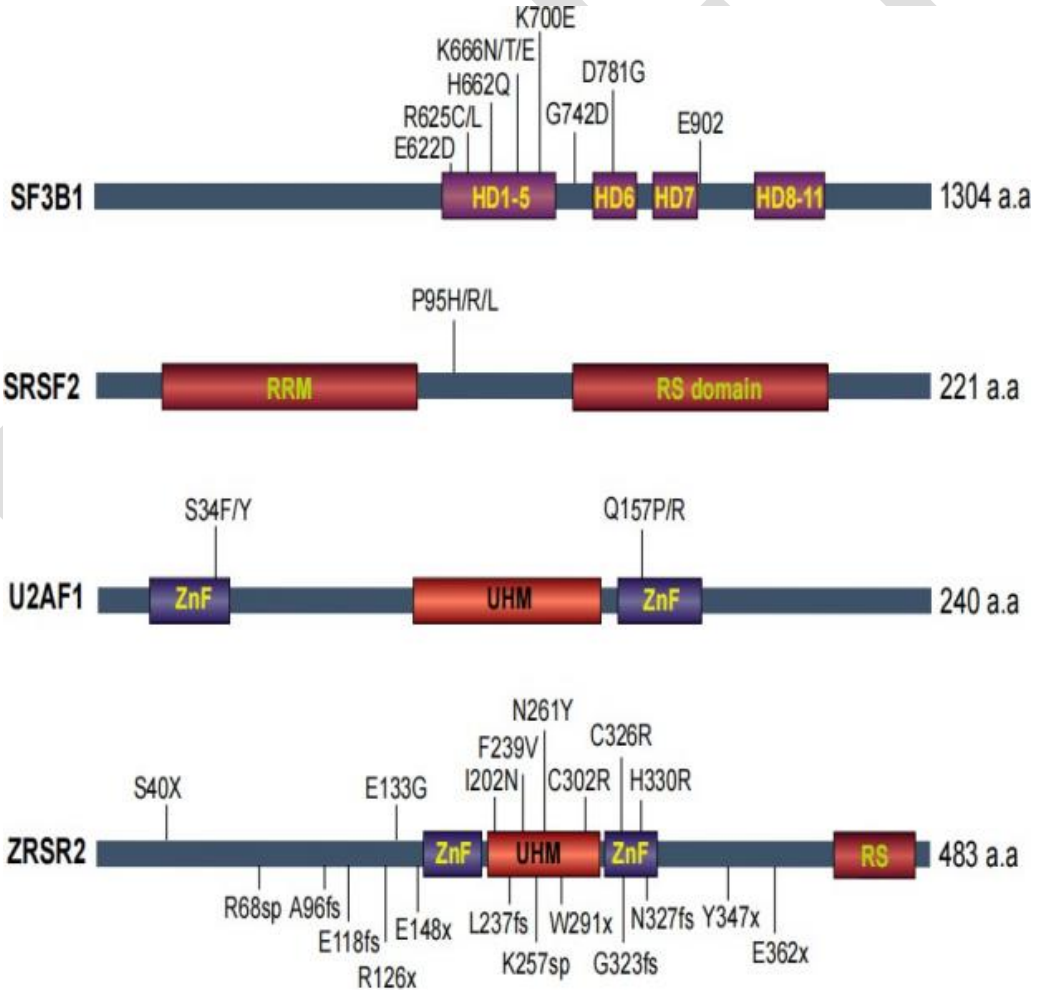

Figure (4): Mutations in the four predominant RNA splicing factors associated with cancer—SF3B1, ZRSR2, SRSF2, and U2AF1—are depicted in the figure, highlighting diverse mutation locations. The mutations are identified within specific domains: HD (heat domain), RRM (RNA recognition motif), RS domain (arginine/serine domain), ZnF (zinc finger), UHM (U2AF homology motif), sp (splice site), and fs (frame shift)

Conclusion

Since the discovery of RNA splicing process and the development of RNA sequencing forty years ago, considerable effort has been devoted to identifying biomarkers associated with cancer and determining how dysregulation of RNA splicing can lead to its development. Various novel therapeutic agents have been developed to address this issue. These include oligonuclotide- based therapies and the use of synthetic introns. Additionally, functional technologies like CRISPR/Cas have been developed that to assist in targeting aberrant RNA splicing.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The raw data that needed to reproduce these findings might be found in the manuscript body and illustrations.

Author' contribution

Study conception and design: DR, GA; figures design: DR; draft manuscript preparation: DR, GA; reviewing the results: DR, GA. The findings of this research were assessed by all authors, who approved the final version of the manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article

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