Significance of alternative splicing in cancer cells

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Abstract

Objective: Alternative splicing can generate various structural and functional protein isoforms. Recently, accumulating evidence shows a relationship between alternative splicing and cancer. Cancer is a complex and chronic disease that involves malignant transformation. In this review, we consider alternative splicing events in relation to the hallmarks of cancer cells, and discuss current therapies to treat cancer-related to alternative splicing.

Data sources: Data cited in this article are from the PubMed and Embase database, primarily focusing on research published from 2000 to 2018.

Study selection: Articles were selected with the search terms "alternative splicing," "cancer cell," "tumor microenvironment," and "therapy."

Results: Alternative splicing plays an important role in tumorigenesis, development, and escape from cell death. Taking this trait of cancer cells into consideration will allow more definite diagnoses of cancer, and allow the development of more effective medicines to intervene in cancer that could focus on controlling alternative splicing or competitively binding to the final products.

Conclusions: Alternative splicing is common in cancer cells. Consideration of alternative splicing may allow different strategies for cancer therapy or the identification of novel biomarkers for cancer diagnosis.

Keywords: Alternative splicing; Cancer cell; Hallmark; Therapy; Tumor microenvironment

Introduction

Alternative splicing, a complicated but highly regulated process in human cells that was first identified by Walter in 1978,[1] allows one gene to code for multiple proteins. Recently, genome-wide applications of next-generation sequencing technology have shown that alternative splicing occurs in more than 90% of human genes.[2-7]

The splicing process is carried out by the spliceosome, which consists of five small nuclear ribonucleoprotein (snRNP) particles (U1, U2, U4, U5, and U6 snRNPs) that assemble at each intron around splice sites. Each splice site consists of a consensus sequence around each exon-intron junction that is recognized by the spliceosome.[8,9] In addition, other sequence components in exons or introns can work as enhancers or silencers and regulate the binding of splicing factors, which can either promote or inhibit the recognition of a given exon by the spliceosome. Some RNA-binding proteins may regulate splicing or the messenger RNA (mRNA) stability of genes, especially for inflammation- and tumor-related genes.[10,11] Among these RNA-binding proteins, two main nuclear RNA-binding protein families, the heterogeneous nuclear ribonucleoprotein (hnRNP) family and the serine/arginine-rich protein (SR) family, often play antagonistic roles in the regulation of exon recognition and act in combination.

After alternative splicing of pre-mRNA, the potential different modes of alternative splicing can be divided into the following categories: exon skipping, intron retention, alternative 5′/3′ donor/acceptor sites, mutually exclusive exons, alternative promoters, and alternative splicing and polyadenylation[12] [Figures 1 and 2].

Different alternative splicing patterns can result in the production of varied transcripts, and these abnormal changes in structure may influence both the gene expression level and translation of the mRNA into protein, giving different functional properties.[14-16]

However, although alternative splicing beneficially allows the production of many varied proteins from a single gene, it can also have negative effects and can play a role in cancer, posing a major challenge for modern medicine. Therefore, this review will focus on the relationship between alternative splicing and the hallmarks of cancer cells.

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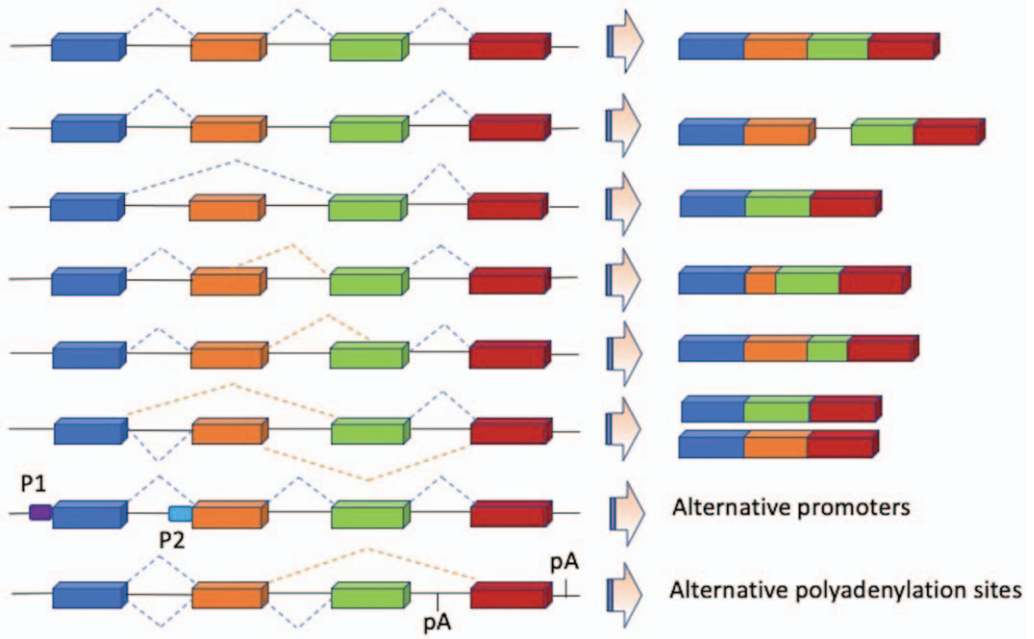

Figure 1: Summary of seven alternative splicing patterns from Blencowe.[12]

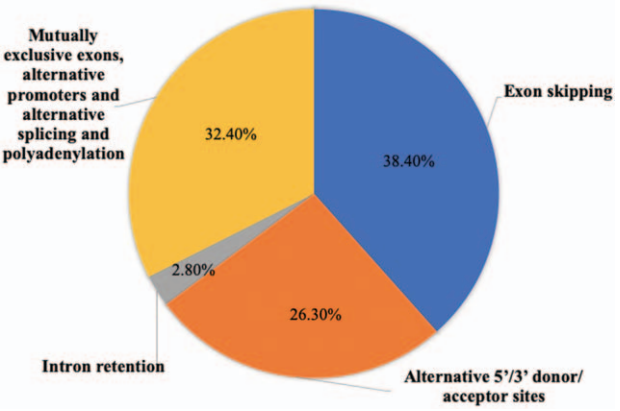

Figure 2: Proportion of alternative splicing events from Sugnet.[13]

Alternative splicing and the hallmarks of cancer cells

Considering that the hallmarks of cancer cells are raised for several years,[17] there has been increasing recognition of the key role played by aberrant splicing in tumorigenesis, cancer progression, and resistance to therapy. The following events correlate with alternative splicing in cancer cells.

Sustaining proliferative signaling

Compared with normal cells, a fundamental trait of cancer cells is sustaining chronic proliferation. Cancer cells can deregulate proliferative signals even without any stimulation induced by a growth factor.

Alternative splicing plays a role in this process. The RAS/RAF/extracellular regulated protein kinases (ERK) pathway, including Kirsten rat sarcoma viral (KRAS) protein, is a key element in most epithelial cell-derived tumors. A positive feedback loop coupling RAS/mitogen-activated protein kinase (MAPK) activation and CD44 variant 6 (CD44v6), which is an alternative splicing variant that includes exon v6 in the cell surface tumor marker clusters of differentiation 44 (CD44), promotes cell proliferation.[18] Once this ability of CD44v6 is utilized by cancer cells, a normal cell may be irreversibly transformed into a malignant cell. CD44v6 overexpression is strongly linked to tumorigenesis and cancer progression in colon cancer, rectal cancer, breast cancer, ovarian cancer, and pancreatic cancer.[19-21] Another conventional signaling pathway is the Wnt/β-catenin pathway. In colorectal cancers, the Wnt pathway promotes a high rate of alternative splicing events.[22] Wnt signaling can also regulate the alternative splicing factor polypyrimidine tract-binding protein 1 (PTBP1). Expression of *PTBP1* is controlled by a transcriptional complex formed by β-catenin, T-cell-specific transcription factor/lymphoid enhancer-binding factor, and nuclear phospho-PKM2 (pSer37), which is phosphorylated by ERK in response to KRAS activation.[23]

Evading growth suppressors

Cancer must also circumvent growth suppression from the actions of tumor suppressor genes that negatively regulate cell proliferation. In hepatocellular tumors, RAS signaling induces AKT activation and subsequent serine/arginine-rich splicing factor 1 (SRSF1)-dependent splicing of the SV1 isoform of Krüppel-like factor 6, which is a cytoplasmic inactive variant of this tumor-suppressing transcription factor.[24] This role can further be deduced from the lack of the phosphorylation of RNA splicing factors including SRSF9, serine and arginine repetitive matrix 1 (SRRM1), SRRM2, transformer 2 homolog

(TRA2B), SRSF10, and CUGBP Elav-like family member 1 in GSK3 knockout cells, which is related to 194 splicing differences in 188 genes.[25] SRSF3 overexpression counteracts p53β-mediated cell senescence by regulating alternative splicing.[26] P53β is a spliceosome of the *TP53* gene, which is a key suppressor of proliferation signaling.

Resisting cell death

Apoptosis, programmed cell death, is a natural barrier to cancer development. The apoptotic machinery consists of upstream regulators and downstream effector components.[27] Caspase-9 (Casp-9) is an initial controller in this program. In lung cancer cells, hnRNPL phosphorylation by activated AKT leads to hnRNPL binding a splice site in Casp-9 pre-mRNA, generating the anti-apoptotic Casp-9b isoform[28,29] and leading to lung tumorigenesis. Casp-9b also participates in Nuclear Factor kappa-B (NF-κB) activation.[30] In hepatocellular carcinomas, SVHB, a specific splicing variant of SVH, is involved in hepatocarcinogenesis. SVHB is not only upregulated but also directly combines with p53 protein to mediate apoptosis. The suppressed expression of SVHB can accelerate the apoptotic program in hepatoma cells.[31] Therefore, there may be the potential to develop a new strategy for tumor suppression by regulating the expression of these genes.

Enabling replicative immortality

Cancer cells have the capacity to generate macroscopic tumors because of the development of unlimited replicative potential.

Telomeres participate in unlimited proliferation by protecting the ends of chromosomes.[32] In the Wnt pathway, human telomerase reverse transcriptase (hTERT), a main component of telomerase, catalyzes telomere production.[33] hTERTα and hTERTβ are the spliceosomes of hTERT.[34] hTERTα is an endogenous inhibitor of telomerase, thereby leading to cell senescence and death, while hTERTβ can trigger mRNA degradation via nonsense-mediated decay resulting from disorderly splicing of the seventh and eighth exons.[33] In myelodysplastic syndromes and melanoma, the hTERTα and hTERTβ expression levels show a substantial difference compared with controls.[35,36]

Inducing angiogenesis

In the normal physiological condition, angiogenesis is generally transient. In contrast, tumor-associated angiogenesis is immortal, can supply nutrients and oxygen, and can evacuate metabolic wastes and carbon dioxide. A well-known angiogenesis inducer is vascular endothelial growth factor (VEGF). There have been multiple studies indicating that VEGF can be regulated by alternative splicing.[37,38] Different splicing methods of the eighth exon of VEGF produce two spliceosomes with opposite functions in angiogenesis. One of these, VEGF165b, competitively binds to the VEGF receptor to inhibit angiogenesis. In human colorectal tumors, VEGF165b downregulation is a marker of poor prognosis.[39] The other VEGF splice variant, VEGF165, is proangiogenic and can be mediated by the transcription factor Wilms tumor 1 (WT1). In the absence of functional WT1, serine-arginine protein kinase 1 (SRPK1) expression and subsequent SRSF1 hyperphosphorylation increase, thereby promoting VEGF165 expression.[40] By contrast, SRPK1 inhibition can affect the progression of prostate cancer by downregulating VEGF165.[41]

Activating invasion and metastasis

Carcinomas arising from epithelial tissues progress to higher pathological grades of malignancy, as reflected by local invasion and distant metastasis. The associated cancer cells typically develop alterations in their shape and attachment to other cells and the extracellular matrix (ECM). The epithelial-mesenchymal transition program broadly regulates invasion and metastasis.[42] In this process, epithelial cells gradually lose their polarity and adhesion and transform into mesenchymal stem cells, which are multifunctional stromal cells that can differentiate into numerous cell types.[43-47] A set of studies documented that CD44 spliceosomes regulate EMT. In breast tumor tissues, the CD44 variant (CD44v) is involved in EMT activity.[48] The overexpression of the CD44 standard isoform (CD44s) is positively related to the EMT status by enhancing Akt signaling to promote the viability of cancer cells.[49] The two spliceosomes of epithelial splicing regulatory protein (ESRP), ESRP1 and ESRP2, regulate EMT.[50] ESRP1 inhibits CD44s by ectopic expression, thereby terminating EMT.[51] In lung cancer cells, decreased ESRP1 expression induces CD44s8–10 overexpression and enhances the potential ability to metastasize.[52] In prostate cancer cells, RNA binding motif 3 overexpression limits CD44s8-10 expression and allows the cells to lose the malignant phenotype and the characteristics of cancer stem cells.[53] The examples above also indicate that the proportions of CD44v and CD44s seem to determine the progress of the tumor. When the proportion of CD44s is high, tumors are always restricted to the organ. In contrast, if the CD44v proportion is high, then the occurrence of tumor invasion and metastasis will dramatically increase.

Reprogramming energy metabolism

Since Otto Warburg first observed that cancer cells have abnormal energy metabolism, the idea that neoplastic disease reprograms energy metabolism for fuel cell growth and division has been increasingly accepted. Even in the presence of oxygen, these cells can refine their glucose metabolism and energy production to glycolysis by limiting energy metabolism, thereby leading to a state called aerobic glycolysis.[54]

Pyruvate kinase (PKM) is the key enzyme in aerobic glycolysis. The two different splicing variants of PKM in enzyme kinetics, PKM1 and PKM2, contain the mutually exclusive exons 10 and 9, respectively.[11,55] PKM1 expression accelerates oxidative phosphorylation in the brain and muscle, while PKM2 expression improves the accumulation of upstream glycolytic regulators to pulse the anabolic metabolism and tumor proliferation.[56,57]

PKM2 overexpression and the excessive accumulation of lactic acid are observed in glioblastoma, lung cancer, multiple myeloma (MM), and hepatocellular carcinoma.[11,58-60] Additionally, increased PTBP1 levels play a role in tumorigenesis, and are associated with a shift in the alternative splicing of the transcript encoding PKM.[61]

Glycolytic fueling is associated with activated oncogenes and mutant tumor suppressors. A recent study revealed an mammalian target of rapamycin complex 1/S6 kinase pathway, leading to the phosphorylation of kinase SRPK2 and subsequent activation of SR protein. This pathway is linked to the U1-70K spliceosome component, and can improve lipogenesis-related transcript splicing to fuel cancer metabolism.[62] In solid tumors, hypoxic regions frequently originate because of a decrease in oxygen availability. Hypoxia-inducible transcription factors (HIFs) can mediate cellular responses to hypoxia.[63] Hypoxia functions in a similar way to oncoproteins, and independently increases the HIF1α and HIF2α levels.[64,65] Parkin can inhibit breast tumor progression by targeting HIF-1α for ubiquitination and degradation.[66]

Evading immune destruction

Cells and tissues are actively and constantly monitored by the immune system, which recognizes and eliminates numerous incipient cancer cells and nascent tumors.[67] Nevertheless, the invasion of immune cells can induce immunoassociated inflammation and subsequent tumorigenesis.[68]

The immune response is classified into innate immunity and acquired immunity. Interferon (IFN) is a pivotal member of the innate immune pathway. Interferon regulatory factor-1 (IRF-1) is a main regulator of *IFN* transcription, but transcriptome sequencing showed that IRF-1 is also associated with alternative splicing in the regulation of growth and differentiation. For instance, carcinoembryonic antigen-related cell adhesion molecule 1 generates variants whenever hnRNP proteins combined with a variable exon 7 can form a complex with promoter-bound IRF-1.[69] hnRNP A1/A2 or SF2/ASF knockdown decreases the inclusion of exons 2 and 3 in IRF-3 pre-mRNA and affects the immunomodulatory functions of human non-small cell lung cancer (NSCLC) cells.[70]

The main effectors of acquired immunity are lymphocytes, which include two main groups, B cells and T cells. T cells are also regulated by alternative splicing of *CD45*.[71] The exclusion of exon cassettes 4, 5, and 6, and the generation of CD45RO,[72,73] also attenuate T cell activation via strong dimerization.[74] hnRNPL-like is directly related to immunoreactive growth hormone mRNA and is more highly expressed in plasma cells than in B cells.[75]

Alternative splicing and the tumor microenvironment

An adverse tissue microenvironment may also cause alternative splicing to become tumorigenic. Mutations and genetic changes alone may not be sufficient to drive cancer as a clinical disease. The tissue microenvironment provides crucial signaling to initiated tumor cells.[76]

As mentioned above, hypoxia is a common situation in solid tumors, and the presence of hypoxia has been linked to malignant progression, metastasis, resistance to therapy, and poor clinical outcomes following treatment. When hepatocellular carcinoma cells were cultivated under hypoxia-mimicking conditions, exon array analysis showed 3059 alternative splicing events in 2005 genes.[77] HIF activation can act through increased expression of CDC-like kinase 1 (CLK1) kinase leading to global hyperphosphorylation of SR proteins and the activation of hypoxia-dependent splice sites in HeLa cells.[78] To some extent, hypoxia also means glucose deprivation. Lack of glucose can cooperate with hypoxia to activate the HIF1α pathway.

Reactive oxygen species (ROS) can have both anti-cancer and tumorigenic effects. Low production of ROS can promote apoptosis, whereas excessive generation of ROS can interfere with signaling pathways and be involved in several pathological conditions, including cancer.[79] In a human gastric cancer cell line (AGS), oxidative stress led to phosphorylation and translocation of splicing factor TRA2B from the nucleus to the cytoplasm. As a consequence, alternative splicing of several variable exons in *CD44*, related to invasiveness, was observed.[80]

Another trait of the tumor microenvironment is hyperosmosis. Stress signals emanating from osmotic shock activate the p38-MAPK pathway via the upstream kinases MKK3 and MKK6 (mitogen-activated protein kinase 3 and 6). Activation of the p38-MAPK pathway induces hnRNPA1 phosphorylation in the nucleus, which is then exported into the cytoplasm and can affect many endogenous alternative splicing events.[81-83]

Growth factors are major regulators of tumor progression, including clonal expansion, invasion across tissue barriers, angiogenesis, and colonization of distant niches.[84] Epidermal growth factor,[85] hepatocyte growth factor,[86] transforming growth factor-β,[87] insulin growth factor,[88] and VEGF are all involved in various alternative splicing events.

The ECM has an important structural support function for cells but is not a static entity. The ECM can be modulated by tumor cells or stromal cells in response to wounding, inflammation, or cancer cell-derived stimuli. Changes in matrix composition, three-dimensional organization, or matrix stiffness communicate with many cell surface receptors[89,90] and result in a signaling response,[91] including changes in alternative splicing. An experiment that remodeled the ECM through activation of extracellular matrix metalloproteinase 3 in mouse mammary epithelial cells induced the expression of splice variant Ras-related C3 botulinum toxin substrate 1b (RAC1b), primarily through release of the repressor hnRNPA1 from an alternative exon.[92] In these cells, RAC1b caused an increase in cellular ROS and stimulated the expression of the transcription factor Snail, which induced epithelial-mesenchymal transition.[93]

Cytokines released by immune cells in the tumor microenvironment can be received by other immune cells and tumor cells of epithelial origin. However, the relationship between them remains to be explored. Interleukin-6 or granulocyte macrophage-colony stimulating factor modulated alternative splicing of BCL2L1 in K562 leukemia cells in favor of the anti-apoptotic splice variant BCL-x(L). Both cytokines required different intronic sequences for their responses, but the underlying molecular mechanisms remained unclear.[94]

Alternative splicing and therapy in cancer

The previous sections of this review describe how both the misregulation of alternative splicing and specific alternative splicing are highly associated with the specificity and severity of disease. Therefore, modulating this process might prevent cancer development and/or alter the course of disease. This could be an exciting strategy for therapy and allow the identification of novel biomarkers for cancer diagnosis.

Common conventional therapeutics involve targeting protein isoforms, expression, and alternative splicing through transacting elements. For example, X-box binding protein 1 (XBP1) is a basic region/leucine zipper transcription factor of the cAMP responsive element binding protein-activation transcription factor (CREB-ATF) family that plays an important prosurvival role in MM cells. Toyocamycin inhibits Inositol-requiring kinase 1a (IRE1a)-induced ATP-dependent XBP1 mRNA cleavage *in vitro*, with no apparent effect on IRE1a autophosphorylation. Therefore, this agent can be used to modulate multiple myeloma (MM) cell death.[95]

However, the therapeutic targeting of splicing factors might affect multiple transcripts, thereby disrupting normal intra-cellular function and generating undesirable side effects. To overcome this challenge, oligonucleotide and RNA-based gene therapies have been proposed. One of the approaches frequently adopted to target splicing is the use of anti-sense oligonucleotides (ASOs). These can be used to target a splice site by blocking it and thereby altering its recognition by the spliceosome, redirecting splicing to an adjacent site.[96] ASOs can also be used to prevent the binding of trans-acting regulatory splicing factors by targeting their binding sites.[97,98]

Some new conceptions in therapy are gradually emerging. Designing a splicing factor to intentionally act on the anti-apoptotic gene BCL-x leads to a high level of its splicing variant, thereby promoting apoptosis and enhancing sensitivity to chemotherapy drugs.[99] In addition, spliceosome inhibitory drugs such as Spliceostatin A or Sudemycins, which target the U2 snRNP component SF3B1,[100] have shown some tumor-cell-specific cytotoxic

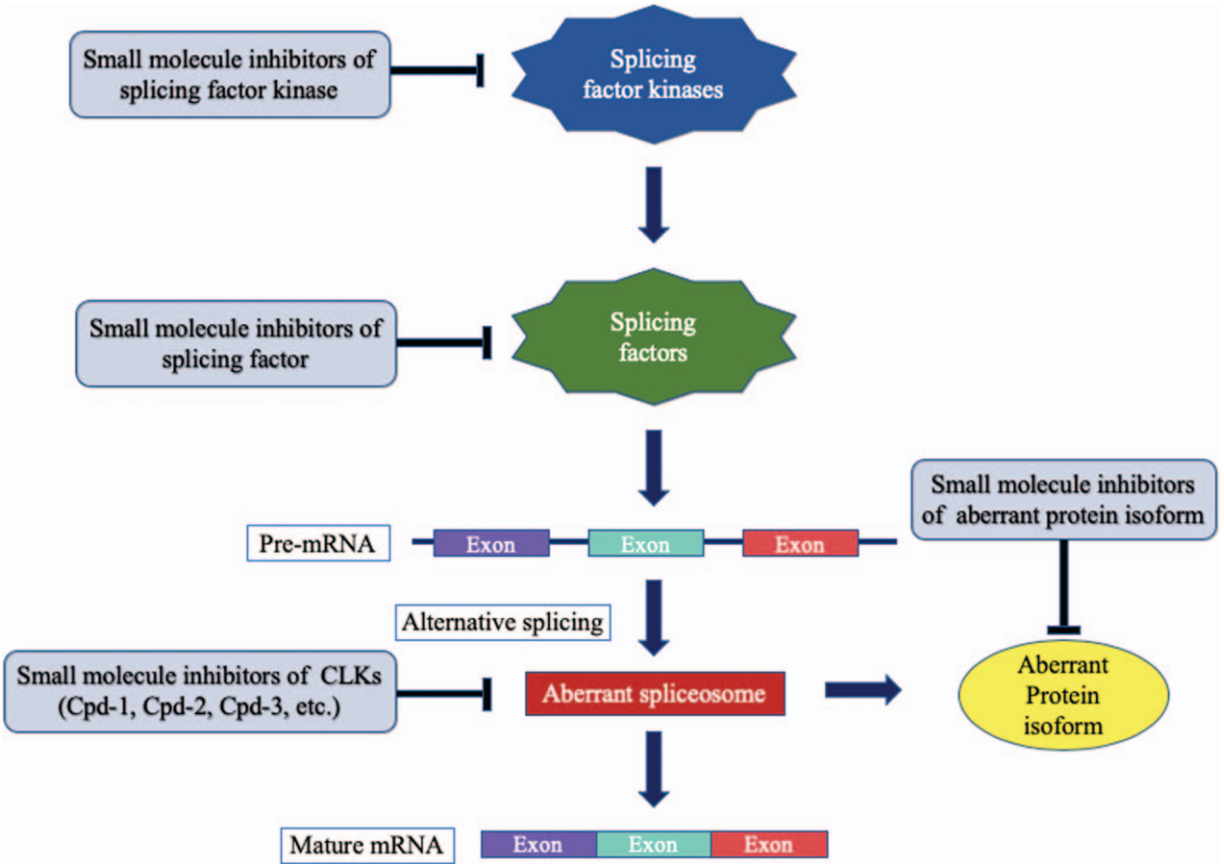

Figure 3: Common alternative splicing therapy methods.

effects in leukemia that were associated with specific changes to alternative splicing.[101]

Conclusions

Alternative splicing plays a significant role in cancer, allowing the malignant progression of initiated tumor cells and contributing specifically to tumor progression. In this article, we reviewed alternative splicing in relation to the hallmarks of cancer cells. In cancer, alternative splicing remains to be comprehensively explored and understood, from tumorigenesis to cancer progression, from intercellular changes to extracellular variation, and from treatment to prevention.

This review describes the improper regulation of alternative splicing and its correlation with disease specificity and severity. Therefore, modifying this process may block the course of disease. This may be an exciting strategy for therapy and the identification of biomarkers for cancer diagnosis, as shown by an attempt to consider the spliceosome as a biomarker in prostate cancer.[102] Common conventional therapeutics involves targeting protein isoforms, expression, and alternative splicing through trans-acting elements [Figure 3]. Although studies on ASO therapies for spinal muscular atrophy and Duchenne muscular dystrophy are still in clinical trials[103,104] and these diseases are unrelated to cancer, this mode of therapy may also prove applicable to the treatment of cancer.

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Conflicts of interest

None.

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