THE ROLE OF ALTERNATIVE SPLICING OF IMMUNE RESPONSE GENES IN CANCER AGGRESSION

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ABSTRACT:

Genetic information is transformed from DNA to RNA and eventually to proteins. The protein phenotype is dependent mainly on the mRNA produced during transcription. Isoform switching is a series of events that occurs after DNA is successfully transcribed into pre-mRNA, which can generate different transcripts from the same gene, leading to both structurally and functionally different and usually pathogenic proteins. Although alternative splicing regulates normal cell stability, it also plays a crucial role in tumor cells under pathological conditions, affecting cancer progression, metastasis, and rapid proliferation by aberrant splicing. Alternative splicing events are linked to at least 15% of all cancers and other fatal diseases. However, reliable quantification of alternative splicing is still hampered by technological constraints, most notably the short-read duration. During an RNA-seq experiment, mRNA is taken from tissue, fragmented, and reverse transcribed into cDNA, which is then amplified and sequenced using high-throughput, short-read sequencing techniques. This review provides insights into various alternative splicing events concerning the immune system, immune escape, and immune therapy and how these events can eventually lead to the development of fatal diseases such as cancer. This review may help identify key biomarkers for a disease's potential prognostic, diagnostic, and therapeutic activities.

Keywords: alternative splicing, isoform switching, cancer therapeutics, immune system, immune therapy.

INTRODUCTION:

In biological systems, the central dogma is the transfer of genetic information from DNA to provide instructions for proteins [(1)]. Replication, transcription, and translation are key processes involved in cell differentiation. However, isoform switching is a series of events that occurs after DNA is successfully transcribed into pre-mRNA structurally and functionally to generate diversity among proteins. This process differentiates cardiomyocytes from other cells, such as hepatocytes. According to a previous study [(2)], approximately 94% of alternative splicing (AS) is estimated to occur in humans. Alternative use of transcript isoforms from the same gene has been proposed as a key feature in malignancies. However, the differential use of gene transcripts between circumstances (isoform switching) has not been thoroughly studied in and across cancer

types. Splicing of precursor mRNA is a complex process regulated by various elements, including the binding of various trans-acting factors to cis-acting elements at the splice sites. Other factors [3], like the stability of splice sites at exon-intron junctions, RNA secondary structure, RNA modification, and structural design of exon/introns, may also serve as prime drivers of AS. Overall, gene expression in a cell is guided by the alternative splicing of non-coding introns and the rearrangement of coding exons in the resultant isoforms.

Although AS regulates normal cell stability, it also plays a crucial role in tumor cells under pathological conditions by affecting cancer progression, metastasis, and rapid proliferation via aberrant splicing. A vast number of alterations occur in the major subunits of the spliceosome machinery [4], marked by a complete loss of function or a change in the open reading frame, inducing the formation of a truncated protein lacking extracellular domains. AS alterations are found to cause 15% of cancers and hereditary diseases [5]. Pathogens mainly attack immune-related genes by dysregulating their expression and inhibiting cell signaling pathways. This helps them to evade tumor suppressor genes [6] and proliferate uncontrollably. Alternative splicing associated with tumorigenesis is marked by changes in the coding or non-coding regions, thereby altering the 5' and 3' splice sites in the precursor mRNA.

A recent study [7] on nine different cancers from TGCA found approximately 224 genes with multiple switches in their isoforms, 58 of which have been reported to be identified in several types of cancers, including pancreatic cancer. According to a study analyzing gene expression levels during aging, approximately 30 % of splicing regulators were responsible for inducing expression variations, that is, aberrant AS in the blood [8]. Elevated intronic retention during aging has also been observed in the prefrontal cortex and Alzheimer's disease [9] in humans during splice switching.

The literature complies with biologically important studies and elucidates how AS dysregulates key immunogenic and immunological functions in organisms. Moreover, this study emphasized that defective AS aberrations tend to be associated with many immune disorders, autoimmune diseases [10], neurodevelopmental disorders [11], and neurodegenerative disorders [5]. Therefore, it also indicates biomarkers for potential prognostic, diagnostic, and therapeutic activities [12] by focusing on changes in alternative splicing and other factors regulating the process. A literature review shows that because of the significant complexity of AS, an extensive study on detecting splicing outcomes in different cellular/experimental contexts is lacking. Nevertheless, the significance of AS in human disorders, along with recent advancements in computational approaches, has proven to be a driving force for in-depth analyses.

1. Mechanism of Alternative Splicing

In cancer samples, the expression levels of cancer-related transcripts depend on the molecular mechanisms of alternative splicing [13]. Alternative splicing (AS) is a process during gene expression in which exons present on the same gene are ligated in different combinations after the excision of intronic regions. It modifies pre-RNA constructs before translation to form a mature mRNA [14]. The macromolecular machinery used for splicing events is the spliceosome [4]. It is a complex of five small nuclear ribonucleoprotein particles (snRNPs). snRNPs, particularly U1, U2, U4, U5, and U6, accumulate around the splice sites of each intron-exon junction [15]. Splice sites are conserved sequences present in the pre-mRNA that are recognized by the spliceosome for cleavage. Enhancers and silencers regulate this process.

Consequently, this process is promoted or inhibited. It is a complex process involving various interacting components, including cis-acting elements present in non-coding regions such as promoters, enhancers, silencers, and trans-acting factors [(2)] (regulatory proteins) that bind to cis-acting elements to control gene expression. Splicing events can occur as shown in the following ways. Table (1).

Table 1: Splicing types and their molecular mechanisms.

Splicing Type	Mechanism
Constitutive splicing	After the removal of introns, most exons are ligated in the order in which they appear in the gene
Mutually exclusive exons	Different splice variants are generated by the ligation of different exons, but they can never occur in the same transcript
Cassette alternative exon	It is generated by either the shuffling of exons or the process of acquiring new exons from the intronic regions
Alternative 3' splice site/Alternative 5' splice site	Transcripts are generated with the inclusion of partial sequences of introns from the 5' and 3' splice sites or exclusive parts of the exon
Intron retention	Transcripts are generated with a portion of an intron along with other exons
Exon skipping	Selected exons are skipped from the mature mRNA (14)

2. Role of alternative splicing in the development of cancer:

Aberrations in cis-acting elements and trans-splicing factors result in altered splicing profiles that facilitate cancer development. Programmed cell death, also known as apoptosis, acts as a barrier to the development of cancer. Regulators and effectors control apoptosis in the upstream and downstream regions. Caspase-9 initiates the apoptotic pathway, which activates caspase-3. In lung cancer, hnRNPL is phosphorylated by the protein kinase B (AKT) enzyme, which initiates the binding of hnRNPL to a splice site in caspase-9 pre-mRNA. This generates the caspase-9b isoform, which is anti-apoptotic and leads to the development of lung cancer. The examination of global splicing profiles among 8700 cases and 32 cancer types from The Cancer Genome Atlas (TCGA) [(2)] recognized that approximately 30 % more alternative splicing is seen in tumor tissues than in non-malignant tissues. Approximately 900 somatic exonic mutations have been identified in over 1800 cancer samples. At least 163 cases have been attributed to intronic retention or exon skipping. Mutations causing intron retention are frequently observed in tumor suppressor genes, which lead to premature stop codon insertion, that is, truncated proteins. Approximately 2000 mutations were found to create novel splice sites after a study of the TCGA dataset. The generation of novel functional splice sites by splice-creating mutations (SCMs) was further validated for 11 candidate genes, including BRCA1 and PARP1.

Moreover, neoantigens formed by SCMs are more likely to be used as potential immunotherapy targets than those created by missense mutations because they are more immunogenic. Pyruvate kinase M (PKM) is an enzyme involved in pyruvate synthesis during the final step of glycolysis. Two mutually exclusive exons, 9 and 10, are

present in the gene encoding PKM and their inclusion results in the expression of either PKM1 or PKM2. This influences the dimerization state of the molecule and its susceptibility to posttranslational modifications. PKM1 is expressed in most adult tissues, whereas PKM2 is overexpressed in the cancer cells. The replacement of PKM2 with PKM1 in cancer cells contributes to tumorigenesis and induces the Warburg effect (aerobic glycolysis of cancer cells). This increased the number of cells. This process is stable in normal cells. Cancer cells undergo abnormal proliferation to grow and rapidly divide. Spliceosome complexes and splicing regulatory factors have been extensively studied in cancer. The snRNPs, hnRNPs, and SR proteins have been demonstrated to behave either as oncoproteins or tumor suppressor proteins in diverse cancer forms, including cervical neoplasia.

In most cancers, including bladder carcinoma, breast carcinoma, breast invasive lobular carcinoma, and endometrial endometrioid adenocarcinoma, the cyclin D1 (CCND1) gene is altered. CCND1 is also involved in cellular growth and differentiation [(16)]. The two isoforms of CCND1, cyclin D1a, and cyclin D1b, have five and four exons, respectively, and are predominantly expressed in many cancers [(17)]. The splicing factors Sam68 and SRSF1 are involved in disturbing U1 snRNP recruitment by binding to intron 4, thereby promoting the production of the cyclin D1b isoform. Moreover, these splicing factors are associated with G/A polymorphism at exon 4 and intron 4 junctions of cyclin D1. On the other hand, cyclin-dependent kinase (CDK4/6) forms a complex with cyclin D1a isoform to activate the phosphorylation of the retinoblastoma protein (Rb), which is a tumor suppressor protein, which as a result, promotes cell cycle progression and aberrantly increases cell proliferation.

3. Alternative splicing in invasion and metastasis of cancer cells:

Invasion refers to the spread of cancer cells to nearby tissues and cells. In contrast, metastasis refers to cancer cell migration to another region of the body far from where it originated. Splicing events highly control the invasive and metastatic abilities of tumor cells. CD44 [(18)] is a receptor protein on the surface of cells involved in the regulation of metastasis. Many factors binding to the upstream region have been reported to target ESRP1 (Epithelial splicing regulatory protein 1) to regulate the splicing of CD44. Snail is a transcription factor that inhibits ESRP1 transcription, thereby disturbing the splicing of CD44 pre-mRNA. The invasive and metastatic abilities of tumor cells rapidly increase in this manner. RON [(19)] is a receptor tyrosine kinase (RTK) that regulates cell motility and induces metastasis. Exon 11 removal from the pre-mRNA of RON has been identified as a contributing factor to the upregulation of delta RON isoform in several types of human cancers, including glioblastomas. This promotes the locomotion and invasion of tumor cells.

Another process that promotes cancer cell metastasis is the formation of new blood vessels, called angiogenesis [(20)]. It involves the migration and growth of endothelial cells (lining the inner walls of the blood vessels) to form new blood vessels. The factor which induces the process of angiogenesis is VEGFA [(21)] (Vascular endothelial growth factor A (VEGFA), which contains eight exons, that induces angiogenesis. Overexpression of pro-angiogenic transcripts of VEGFA occurs when the proximal 3' splicing site is selected in exon 8. However, the distal 3' splicing site enhances the formation of anti-angiogenic VEGFA-b transcripts, and their expression is suppressed in tumor cells [(17)].

SR protein [(22)] family acts as a splicing factor by activating and repressing splicing events. Alternative splicing of vascular

endothelial growth factor A (VEGFA) is associated with the recruitment of SR proteins. For example, the expression of SRSF protein kinase 1 (SRPK1) in normal cells is inhibited by transcription factor WT1, which binds to its promoter. Overexpression of SRPK1 induces more significant phosphorylation of SRSF1, which in turn increases metastasis by increasing cancer growth and regulating alternative splicing of VEGFA [17].

4. Alternative splicing in immune response and immune escape:

When a foreign substance (antigen) enters the body, an immune response is activated to protect the body. MyD88[23] is involved in immune cell signaling. MyD88s control the production of inflammatory factors in macrophages by inhibiting innate immune activation. The connection between alternative splicing and innate immune response can be determined by studying how splicing factors, including Eftud2, are regulated by MyD88s. Furthermore, MyD88 signaling is inhibited when there is an increased short subtype of MyD88 in the absence of splicing factors, such as SF3A1, SF3A2, SF3A3, or SF3B1, which are important for the generation of the long subtype in TLR4 (Toll-like the TLR) signaling pathway, which is essential for initiating an innate immune response.

Additionally, a soluble isoform induced during the inflammatory response can be formed by inserting a 144-base pair between exons 2 and 3, resulting in a truncated protein. The diversity of immunoglobulin isotype IgE in B cells [24] is regulated by splicing. IgE also exists in secreted and membrane-bound subtypes. HuR is a spliced protein that effectively regulates alternative splicing events in B lymphocytes. The absence of this protein results in B-cell death via abnormal class switching. CD45 isoforms (with 34 exons) during T-cell development vary at different stages. Exons 4, 5, and 6 are alternatively spliced. The splicing factor hnRNP LL, expressed in activated T-cells, is responsible for the exclusion of exons 4 and 6. CD45 spliced transcripts are critical for the development and function of T cells, as elevated expression levels of hnRNP LL favor CD45 splicing. This results in an increase in the molecular weights of the subtypes [25].

Immune escape is when a virus or any other foreign invader dodges the immune system to mutate and infect the body. Interleukin-33 [26] (IL-33) is a cytokine receptor that binds to the suppression of tumorigenicity 2 (ST2) factor and has three isoforms: ST2L, sST2, and ST2V. IL-33, when released, activates the ST2L/IL-1 RacP heterodimer on the surface of immune cells, which increases the transcription of inflammatory genes by activating various cytokines and kinases. Normally, ST2 isoform sST2 acts as a bait receptor to bind IL-33, which blocks the activation of the IL-33/ST2L signal [17]. The IL-33/ST2L axis facilitates cancer development. It has been observed that the IRAK4-S isoform, also known as interleukin 1 receptor-associated kinase 4, is predominantly expressed in healthy hematopoietic cells. This indicates that it may play a crucial role in maintaining these cells' health and proper functioning. Reduced binding of IRAK4-S to MyD88 reduces NF-ÎºB activation potential. In contrast, the IRAK4-L isoform activates NF-κB [27] by binding to MyD88, as reported in cancers, including myelodysplastic syndrome and acute myeloid leukemia.

Healthy cells control proliferation with growth suppressors such as TP53, PTEN, and RB. Cancer cells tend to multiply uncontrollably by evading the growth suppressors. When these suppressor genes are activated, the cell cycle stops, or apoptosis is induced. Cancer cells suppress the expression of these genes. They create mutations in the gene so that they cannot be activated or completely lost. The predominant cause of human tumors is

mutations or deletions in the TP53 gene. The oncogenic inactive splice variant SV1 of the tumor-suppressing transcription factor KLF6 (Krüppel-like factor 6) in liver cell tumors is stimulated by RAS signaling through the activation of AKT kinases and splicing factors such as SRSF1. This was due to 194 splicing differences in 188 genes occurring in other splicing factors, such as SRSF9, SRRM1, SRRM2, TRA2B, SRSF10, and CUGBP, which were not phosphorylated/activated in this case. Moreover, SRSF3 is upregulated in cancers, and its overexpression neutralizes the normal effect of P53β, a spliceosome of the TP53 gene involved in suppressing the signaling pathways involved in cell proliferation[9].

The first line of defense in the immune system is innate immunity [28], which protects the body against antigens. Cells that link innate immunity with adaptive immunity by presenting antigens on their surfaces are called dendritic cells. They initiate the innate immune response. On normal cells, the expression of KIR receptors on natural killer cells facilitates the MHC-I (major histocompatibility complex) interactions. KIR splicing affects the normal functioning of naturally killed cells involved in innate immunity. The IFN signaling pathways in host cells are associated with regulating innate immunity. Splicing events of ISGs (IFN stimulating genes (ISGs) can cause aberrant changes in the resulting transcripts that affect the normal activity of their proteins against viral attack inside the body. The IFN response can be repressed by the zinc finger RNA-binding protein (ZFR), which is a key regulator of ISG splicing. Truncated ZFR is observed in many monocytes, whereas full-length ZFR is expressed in macrophages as a protector against the responses produced by IFN signaling. Another antiviral gene, SAT1, in infected cells has increased exon 4 selection during alternative splicing, which facilitates infection.

Humoral immunity [28] is adaptive immunity in which B cells and plasma cells produce antigens specific to foreign materials found in the humor (body fluids).

B-cells [24] -produced antibodies facilitate humoral immunity. Isotype IgM is the first immunoglobulin (antibody) produced by activating the immunoglobulin gene in B cells. Other isotypes of IgG, IgA, IgD, and IgE are also produced by irreversible rearrangement and recombination of the Ig gene. On the other hand, alternative splicing of an mRNA transcript of the Ig heavy chain gene IGH is involved in converting IgM to IgD. The splicing of mRNA and its export from the nucleus to the cytoplasm is regulated by the splicing protein HuR [29]. The absence of B cells disturbs the normal proliferation and differentiation of B lymphocytes. HuR induces changes in the resulting Ig isotypes by controlling the splicing events of many genes in B-cells; HuR induces the changes in the resulting Ig isotypes.

To meet their own energy needs, cancer cells [30] tend to cause a metabolic shift by altering the normal metabolism of the cells to promote rapid growth of the tumor cells. Unlike in normal cells, in aerobic glycolysis, glucose is converted into lactic acid, known as the Warburg effect. However, this process yields less energy than normal aerobic glycolysis, which produces large amounts of ATP. Cancer cells use this method because only a few ATP molecules are produced; therefore, the remaining glucose is used to produce intermediates essential for tumor growth. Another reason is that the lactic acid produced favors tumor invasion by decreasing extracellular pH [25]. During cell division, normal cells utilize large quantities of stored ATP produced by respiration and undergo the Warburg effect by producing lactic acid to maintain the raised intracellular pH. Once cell division is completed, cells return to normal respiration-based ATP production. However, tumor cells have an elevated intracellular pH of 7.4, owing to the

upregulation of various acid-loading transporters. These cells undergo the Warburg effect [31] to ensure ATP synthesis, even in the absence of oxygen, which is common during the overgrowth of tumor cells, where the cells can exceed the oxygen supply. It helps in tumor invasion by lowering pH through the release of lactic acid. Moreover, because only a few ATP molecules are produced, the remaining glucose is used to produce intermediates essential for tumor growth. Thus, both NPCs and cancer cells utilize these effects for various purposes.

Hypoxia (low oxygen levels) is common during tumor progression [32]. It alters metabolism and induces metastasis. Several adaptive mechanisms have been stimulated in response to hypoxia [33] for cell survival, as hypoxia is an essential component of the upholding metabolism. Alternative splicing induced by hypoxia influences tumor initiation. Heterodimeric transcription factor subunits, HIF-1α and HIF-2α, are directly targeted by growth factors such as VEGF for angiogenesis. Under normal oxygen levels, these isoforms of VEGF are stimulated, inhibiting angiogenesis, whereas tumor hypoxia causes the overexpression of these isoforms, which encourages angiogenesis.

To generate large amounts of ATP (energy), cells undergo a metabolic pathway (glycolysis) in which two molecules of glucose are converted into two molecules of pyruvate by a series of reactions catalyzed by enzymes. PK (pyruvate kinase) is an enzyme responsible for the final step (phosphoenolpyruvate). Two isoforms of pyruvate kinase muscle isozyme (PKM) are produced in muscles: PKM1 and PKM2. The splicing factors hnRNPA1, hnRNPA2, and SRSF3 regulate splicing events; hence, the two isoforms are produced by the mutually exclusive alternative splicing of exons 9 and 10. PKM2 is overexpressed in tumor cells, promoting aerobic glycolysis to generate energy for highly proliferating cells. This could be used as a therapeutic target for cancer treatment. Substitution of PKM2 with PKM1 (responsible for aerobic glycolysis in normal cells) can decrease lactic acid production, thereby inhibiting tumor progression.

Continuously proliferating cells, such as tumor cells, require more energy to meet their needs; therefore, they tend to utilize all available nutrients in the body. Fructose (an isomer of glucose) was used to maintain the required carbon voltage in cells. Keto hexokinase (KHK) activates fructose metabolism via phosphorylation. KHK-A and KHK-C are splice variants of KHK. Several conditions are associated with the selection of the KHK variants. For instance, in the liver and intestine, KHK-C is overexpressed and is associated with cancer development. Glycerol-3-phosphate and glyceraldehyde-3-phosphate are produced during fructose metabolism. They help synthesize essential energy molecules such as phospholipids and triglycerides.

Fatty acid metabolism [25] is crucial because it contributes to multiple functions inside the cell, mainly for energy storage. Fatty acids (FAs) enter cells via multiple transporters. It is important for fatty acids first to be transformed into acyl-coenzyme A (acyl-CoA) by an enzyme called acyl-coenzyme A synthetase (ACS), located on the surface of the mitochondria and endoplasmic reticulum (ER), forming long-chain acyl CoA. ACSL4, the fourth splice variant of the long-chain acyl-CoA synthetase (ACSL) family, is a valuable biomarker because it is upregulated in many cancers, including the liver and breast. It also aids tumor growth.

5. Alternative splicing and immune therapy:

Focusing on the splicing events of immune genes that result in the generation of cancer-related isoforms, many therapies [12] can help remove these variants and treat particular conditions. Natural compounds can effectively regulate alternative splicing, as

has been studied in the past decade. Pladienolides and herboxidiene [25] are compounds used to regulate the function of the spliceosome U2 snRNP. This occurs when the fundamental subunit of the spliceosome, SF3B1, is directly targeted by natural compounds obtained from bacteria (Streptomyces spp. and Pseudomonas spp.). Spliceostatin A (SSA) is a chemical compound that can cause changes in splicing events by stopping the spliceosome interaction of U2 snRNA with the sequence present at the branching site of pre-mRNA and preventing SF3B1 from interacting with pre-RNA. This suggests that natural compounds can be used to develop other stable chemical compounds that can influence alternative splicing.

Additionally, proteins involved in regulating alternative splicing can be used as therapeutic targets. Splicing regulatory proteins is essential, as they activate and repress splicing events. Derivatives of indole produced by various bacteria have been confirmed to target splicing regulatory proteins (SR), yet no specific explanation to confirm the mechanisms involved has been deduced. Therefore, we concluded that SR kinase inhibition is a potential therapeutic approach.

Oligonucleotides are short nucleotide sequences with a vast range of applications in research. Contemporary research has brought attention to potential therapies using splice-switching oligonucleotides (34) (SSOs), which can alter isoforms. SSOs have modified nucleotide sequences that disrupt splicing by base-pairing a pre-RNA with specific sequences on RNA, including 5’ and 3’ splice sites. This hinders the inhibition of other regulators that bind RNA. This regulates splicing events by bringing back or closing the open reading frame of the target protein.

6. Abnormally spliced isoforms in autoimmune diseases:

Systemic lupus erythematosus (SLE) [34] is a form of lupus that predominantly occurs in women. It causes inflammation and tissue damage in specific regions of the body, including the kidneys, blood vessels, joints, and skin. Type I interferons (IFN) are signaling proteins known as cytokines that have immunoregulatory functions. The defective IFN-I signaling pathway is a key indicator of SLE. Genetic factors can also disrupt splicing. A genome-wide association study has identified 50 loci associated with SLE [35].

The interferon regulatory transcription factor IRF5[36] is predominantly expressed in SLE and is linked to the expression of alternative untranslated exons 1 B (v2, v9, v10). IRF5 contains nine coding exons. Upon alternative splicing, it can produce various transcripts by the differential selection of coding and non-coding exons present in pre-mRNA, labeled V1–V11. This was due to the formation of a new splice site in the pre-RNA region at the junction of the exon and the intron. In SLE, genetic factors such as SNPs may also influence the formation of disrupted isoforms by creating new 5’ and 3’ splice sites. In particular, a new splice donor variant at the 5’ splice site was formed by SNP (T>G) rs2004640 at 1 B. However, poly-A in the 3’ UTR of exon 9 was removed by SNP (G>A) rs10954213.

SLE was reported to be associated with LILRA2 (Leukocyte Immunoglobulin Like Receptor A2) related polymorphisms in some populations of Japan in which normal splicing was aberrated at the junction of intron 6 and exon 7 by the SNP rs2241524 (G>A). SRSF1[10] is a splice regulatory protein that binds to pre-mRNA. The normal RasGRP1 isoform (functional) is under expressed in SLE T lymphocytes. A mutant isoform without exon 11 is frequently found, and its inclusion is ensured by binding to SRSF1. In healthy T lymphocytes, the

functional RasGRP1 isoform is produced when SRSF1 is degraded using siRNAs.

Rheumatoid arthritis (RA)[37] is an inflammatory disorder that usually affects the tissues of joints and is characterized by painful swelling, causing bone damage. It is caused by several aberrant alternative splicing events, including the glycoprotein CD44[18], which facilitates proliferation and metastasis. It contained 20 constant and variant exons. The inclusion of constant exons in CD44 represents its regular form, primarily observed in the blood stem cells. The alternative splicing of variant exons, glycosylation, and glycosaminoglycanation generates many transcripts (CD44v). The most common variants in rheumatoid arthritis are CD44v4, CD44v6, CD44v7, and CD44v8. The pathogenesis of rheumatoid arthritis is influenced by multifunctional cytokine (necrosis factor-alpha (TNF-α ()[27], which is released by immune cells and is involved in controlling cell proliferation. The regulation of TNF signaling via TNFR1 and TNFR2 elicits many cellular responses. Soluble forms of TNFR (sTNFR) are generated via enzymatic proteolysis. Patients with RA and SLE have high levels of soluble sTNFR. Moreover, in RA, DS-TNFR2, the cytoplasmic domain of soluble TNRF2, is formed by excluding exons 7 and 8. TNRF2 blocks apoptosis by antagonizing the TNF-α signaling pathway. This is a key indicator of the severity of RA in patients.

Alternative splicing of a human endothelial factor with seven introns and eight exons generates various transcripts, including VEGF−A121, VEGF−A145, VEGF−A165, VEGF−A189, and VEGF−A206[34]. Overexpression of VEGF121 and VEGF165 on the surface of muscle cells, macrophages, and fibroblasts affects the synovial tissues of patients with RA. The isoform encoded by exon 7 of VEGF165 binds heparan sulfate, while in the VEGF121 isoform, this region is absent, which changes its spreading ability compared to that of VEGF165.

Another glycoprotein, fibronectin[38] (Fn), controls overall cell behavior and tissue repair. It is found at multiple locations in the body. Upon alternative splicing, Fn generates several isoforms, mainly type III connecting segment (IIICS), extra domain A (EDA), and extra domain B (EDB), which play essential roles in the body. Increased levels of synovial extra-domain A isoform characterize increased joint destruction. This biomarker has been used to determine the prognosis of RA patients.

Conclusion:

In conclusion, alternative splicing is a complex process in which various interacting components are involved, including cis-acting elements present in non-coding regions, such as promoter silencers and trans-acting factors that bind to cis-acting elements to control gene expression. In cancer, aberrations in cis-acting elements and trans-splicing factors alter splicing profiles. The splicing of key genes initiates the development and progression of various cancers, such as bladder carcinoma, breast carcinoma, and pancreatic cancer. These events strongly control the invasive and metastatic abilities of tumor cells. Hence, by focusing on the splicing events of key immune response genes that result in the generation of cancer-related isoforms, new therapeutic biomarkers can help inhibit the progression of cancer by inhibiting pathogenic isoforms.

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دور عملية تبدل النسخ لجينات الاستجابة المناعية في شراسة السرطان

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تتحول المعلومات الوراثية من DNA الى RNA وفي النهاية الى بروتينات. يعتمد النمط الظاهري للبروتين بشكل اساسي على mRNA المنتج اثناء عملية الاستنساخ . ان عملية تبدل النسخ هي عبارة عن سلسلة من الاحداث التي تجري بعد عملية نسخ DNA الى نسخة اولية من mRNA والتي يمكن ان تعطي عدة طرز من mRNA من نفس الجين , والتي تؤدي الى انتاج بروتينات مختلفة وظيفيا وتركيبا وعادة ما تكون مرضية. على الرغم من أن تبديل النسخ تنظم استقرار الخلايا الطبيعية، إلا أنها تلعب أيضًا دورًا حاسمًا في الخلايا السرطانية في ظل الظروف المرضية، مما يؤثر على تطور السرطان، والورم الخبيث، والانتشار السريع عن طريق الربط الشاذ. ترتبط أحداث تبديل النسخ بما لا يقل عن ١٥% من جميع أنواع السرطان والأمراض القاتلة الأخرى. ومع ذلك، فإن التحديد الكمي الموثوق للربط البديل لا تزال تعوقه القيود التكنولوجية، وأبرزها مدة القراءة القصيرة. أثناء تجربة RNA-seq، يتم أخذ mRNA من الأنسجة، وتجزئته، ونسخه عكسيًا إلى cDNA، والذي يتم بعد ذلك تضخيمه وكشف تسلسلاته باستخدام تقنيات التسلسل عالية الإنتاجية وقصيرة القراءة. توفر هذه المراجعة نظرة ثاقبة لمختلف أحداث الربط البديلة المتعلقة بالجهاز المناعي، والهروب المناعي، والعلاج المناعي وكيف يمكن أن تؤدي هذه الأحداث في النهاية إلى تطور أمراض مميتة مثل السرطان. قد تساعد هذه المراجعة في تحديد المؤشرات الحيوية الرئيسية لمتابعة المرض والجوانب العلاجية والتشخيصية المحتملة للمرض.