Review

Expression of mRNA Isoforms in Health and Diseases

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Review

Expression of mRNA Isoforms in Health and Diseases

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Abstract: Cellular gene expression varies in different physiological and pathological conditions. Analysis of differential gene expression enables researchers to understand the cellular changes associated with physiological or pathological conditions. During gene expression analyses, researchers calculate the transcripts expressed from a gene under that gene's name, assuming only a single mRNA is expressed by that gene. However, this assumption is biologically inaccurate, but it is often preferred to avoid the complex analyses of isoforms. Most mammalian genes express more than three mRNAs, which encode various proteins or act as noncoding RNAs. We have previously addressed the molecular basis mRNA isoform formation and detection strategies. In this review article, we have discussed the physiological and pathological roles of mRNA isoforms. Any specific cell may express different isoforms of mRNA from a specific gene depending on its differentiation state. Different isoforms can be expressed from a single gene at various stages of development and during aging. The same cell may also express mRNA isoforms related to pathological conditions. Summarized findings highlight the importance of detecting mRNA isoforms over conventional gene identification.

Keywords: gene expression; mRNA isoforms; cell differentiation; organogenesis; aging; molecular pathology; mRNA isoforms in diseases

1. Introduction

mRNA transcript variants are the diverse RNA isoforms derived from a single gene locus. These isoforms arise due to alternative transcription start sites (TSS), alternative splicing of precursor mRNAs (pre-mRNAs), and alternative transcription termination and alternative polyadenylation sites (APS). Alternative TSS and APS result in variation in the 5′-end and 3′-end of mRNA, respectively; however, alternative splicing can alter any part of the pre-mRNA [1]. Eventually, mRNA isoforms vary in their coding sequencing in the amino-terminal, carboxy-terminal, or other functional domains and encode proteins with diverse functional domains or form noncoding regulatory RNAs [2,3]. Enabling a single gene to encode multiple protein isoforms or non-coding RNAs, the mRNA transcript variants fine-tune cellular signaling, differentiation, and adaptation to environmental cues [4,5]. Tissue cells generate selective mRNA isoforms that help cells adapt to dynamic physiological and pathological conditions [6,7]. This transcriptomic plasticity is essential for developmental programs, tissue-specific functions, and the decline of functions during aging. Thus, dysregulation of mRNA isoform expression may disrupt molecular homeostasis, contributing to various diseases [8,9].

In embryonic development and organogenesis, they facilitate precise spatiotemporal gene expression, impacting heart, brain, and placental development [10]. Tissue-specific splicing events and APS create diverse mRNA molecules with variable 3' untranslated regions (UTRs), influencing mRNA stability, localization, and translation efficiency [11]. In physiological contexts, isoforms orchestrate critical processes such as cell lineage specification, immune response modulation, and

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neuronal plasticity [12]. Tissue-specific splicing factors and RNA-binding proteins guide the production of isoforms tailored to distinct cellular niches, ensuring precise spatiotemporal control over gene expression [13]. However, perturbations in RNA processing machinery due to mutations in spliceosome components, epigenetic alterations, or aberrant expression of regulatory RNAs can generate pathogenic isoforms [14]. These dysregulated isoforms often exhibit gain-of-function, dominant-negative, or neomorphic properties that subvert normal cellular checkpoints, fostering disease progression [15]. During aging, changes in splicing patterns, increased errors, and alterations in chromatin structure disrupt mRNA isoform production [16,17]. Accelerated transcription and splicing increase the chance of erroneous splicing events, affecting genes involved in DNA repair, apoptosis, and muscle homeostasis, contributing to cellular decline [18]. The activities of retrotransposons and the quality control mechanisms of RNA also influence these changes [19,20].

The pathological influence of mRNA isoforms spans cancer, neurodegenerative disorders, cardiovascular diseases, and metabolic syndromes [21]. Aberrant isoforms may enhance oncogenic signaling, promote protein aggregation, or impair contractile function in tissues, depending on the disease context [22,23]. Emerging evidence also implicates these isoforms in immune evasion, drug resistance, and intercellular communication, underscoring their systemic impact [24]. Critically, advances in long-read sequencing and single-cell transcriptomics have unveiled previously unrecognized isoform-level alterations, redefined molecular classifications of diseases, and revealed novel biomarkers [25]. Thus, studying the mRNA isoforms is essential for advancing our understanding of gene expression and its implications for health and diseases [26]. In the following sections, we have discussed the importance of studying isoforms in normal physiology, disease pathology and the potential for translational research [27]. This process enables a single gene to produce multiple transcript variants, allowing for fine-tuned regulation of cellular processes, including signaling, metabolism, and immune responses. However, dysregulation of mRNA isoforms can disrupt these processes, leading to pathological outcomes across a wide range of diseases [28].

2. Physiological Roles of mRNA Isoforms

Although most mammalian genes express mRNA isoforms, this area has been minimally explored due to the complexity of detecting and characterizing the isoforms. Our recent efforts have been targeted at revisiting this field of molecular biology [9,29,30]. mRNA isoforms diversify the gene's protein expression and cellular functional capacity. In the following section, we have discussed the physiological importance of mRNA isoforms during development, organogenesis, and aging.

2.1. mRNA Isoforms in Development and Organogenesis

The role of mRNA isoforms in development and organogenesis begins at fertilization, where maternal transcripts deposited in the oocyte guide early embryonic processes [31]. Following fertilization, the maternal-to-zygotic transition (MZT) marks a pivotal shift in gene regulation as zygotic genome activation (ZGA) replaces maternal control [32]. During this transition, alternative splicing generates specific mRNA isoforms critical for embryonic development [33]. In zebrafish embryos, genes like *Pou5f1* (*Oct4*), *Sall4*, and *Dnmt1* express isoforms associated with pluripotency and DNA methylation, essential for ZGA and early cell fate decisions [34]. Similarly, isoform changes in genes like *F11r* and *Magi1* alter coding sequences to facilitate cell-cell interactions during ZGA [35].

In mice, RNA-binding proteins such as CSDE1 undergo isoform switching during ZGA, characterized by exon skipping that impacts maternal transcript clearance [36,37]. Splicing factors like SF3B1 and RBFOX2 also play crucial roles in generating isoforms that regulate pluripotency and lineage specification [38]. These splicing events are tightly coordinated with chromatin remodeling processes mediated by histone variants like H2A.Z, which destabilize nucleosomes to enable transcriptional activation [39,40]. Transcriptome-wide analyses in zebrafish and mouse embryos revealed the dynamic changes in isoforms that regulate tissue-specific gene expression [41]. As the

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embryo develops through gastrulation and organogenesis, waves of isoform switching correlate with the formation of major organs such as the heart and neural tube [42]. Alternative splicing of genes like *RBM20* and *NOVA2* generates isoforms essential for cardiac development and neural differentiation [43]. In addition, transcription factors such as SRF influence cardiac-specific gene expression by regulating transcript variants critical for myocardin-mediated developmental pathways (44-46). Variants of transcription factors such as NR5A1, RUNX, and BAD are involved in adrenal and gonadal development [47]. It has been demonstrated that NR5A1 isoforms are expressed in gonadal precursor cells and regulate the differentiation of the adrenal and gonadal tissues. Mutations or dysregulation of NR5A1 can lead to disorders of sex determination and adrenal insufficiency [48,49].

Epigenetic mechanisms further regulate the generation of mRNA isoforms during organogenesis. Histone acetyltransferases (HATs) like p300/CBP deposit H3K27 acetylation marks at active enhancers, facilitating tissue-specific gene activation [50,51]. Conversely, histone deacetylases (HDACs) compact chromatin to repress transcription in specific sites [52]. DNA methylation complements these processes by silencing non-essential genes, while demethylation activates enhancers critical for tissue identity [53,54]. Throughout organogenesis, APS generates mRNA isoforms with variable 3′UTR lengths that impact post-transcriptional regulation [55]. For example, APS of Hmga2 produces shorter isoforms with reduced regulatory complexity but increased translational efficiency, supporting rapid protein synthesis during development [56,57].

As the embryo matures into a fetus, mRNA isoforms continue to undergo complex developmental programs across tissues [58]. Aberrant expression or processing of these isoforms can lead to developmental abnormalities such as neurodevelopmental disorders or congenital defects [59]. Moreover, aberrant splicing events caused by synonymous or intronic variants can activate cryptic splice sites, leading to exon truncation or misregulation of developmental genes [60]. For instance, mutations affecting splicing regulators, such as FOXP4 or frameshift variants in HMGB1, disrupt normal gene expression [58]. The frameshift variants in the HMGB1 protein have been shown to alter phase separation and nucleolar function, resulting in rare syndromes such as brachyphalangy, polydactyly, and tibial aplasia syndrome, underscoring the role of mRNA isoforms in proper organogenesis [58,61,62].

2.2. mRNA Isoforms Regulating Physiological Functions

mRNA isoforms are fundamental to normal physiology after birth, contributing to tissue-specific gene expression, protein diversity, and cellular adaptation to environmental stimuli [63]. Through mechanisms such as alternative splicing and alternative polyadenylation, mRNA isoforms allow a single gene to produce multiple protein variants with distinct functions or regulatory properties [9,64]. By allowing a single gene to generate multiple transcript variants, mRNA isoforms diversify protein functions and regulate cellular processes [65]. This process is essential for maintaining the specialized functions of different tissues and organs [66].

Alternative splicing, a major driver of transcript diversity, dynamically regulates gene expression across tissues. For instance, the CACNA1C gene, which is involved in cardiac function and blood pressure regulation, generates over 10,000 splice variants, with some isoforms specific to smooth muscle and others to cardiac tissue [67,68]. In the nervous system, splice variants of neurotransmitter receptors and ion channels play critical roles in synaptic plasticity and neuronal function [69]. The Drosophila Dscam gene exemplifies the extreme diversity generated by alternative splicing, producing over 38,000 isoforms through the alternative splicing of 95 variable exons that contribute to neural connectivity [70,71]. RNA-binding proteins (RBPs) are key regulators of alternative splicing and other post-transcriptional processes. RBPs such as SRSF1 and hnRNPs influence exon inclusion or exclusion, shaping protein structure and functionality critical for cell growth, differentiation, and metabolism. These proteins also regulate mRNA stability, localization, and translation efficiency, ensuring precise control of cellular processes in response to physiological changes [72]. RNA modifications further enhance regulatory complexity. For example, N6-

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methyladenosine (m6A) modifications influence mRNA structure, stability, splicing, export, translation, and decay [73,74]. During stress conditions like heat shock, m6A modifications in the 5′ UTR of HSP70 mRNA promote cap-independent translation to support cellular adaptation [75,76].

APS generates mRNA isoforms with variable 3′ UTRs, altering regulatory elements such as microRNA binding sites and RNA-binding protein interactions [77]. This mechanism enables tissue-specific gene expression and modulates protein abundance in response to environmental cues [78]. It has been reported that APS increases protein levels in skeletal and cardiac tissues while reducing neuronal gene expression through intronic cleavage [79]. The generation of mRNA isoforms is controlled by tissue-specific regulation of pre-mRNA processing. TBX20 transcription factor isoforms are expressed selectively in tissues like the aorta, coronary artery, testis, pituitary gland, and heart [72]. Such specificity underscores the significance of mRNA isoforms in regulating gene expression across various organ systems.

2.3. mRNA Isoforms in Aging

The aging process profoundly impacts the generation of mRNA isoforms, driving changes in gene expressions and cellular functions. As organisms age, alternative splicing patterns shift, altering tissue-specific expression and function of critical genes [80]. Nearly 49,869 splicing events have been identified during human aging that can affect DNA repair, apoptosis, and RNA processing [81]. These changes are often accompanied by an imbalance in transcript length, with shorter transcripts linked to inflammation and functional decline, while longer transcripts are associated with increased lifespan [82].

Epigenetic changes play a significant role in altering mRNA isoform expression during aging. The loss of histone H3K36 methylation exposes cryptic promoters, leading to the production of non-canonical transcripts that may disrupt gene regulation and limit lifespan [83,84]. RNA-binding proteins (RBPs), which regulate splicing, mRNA stability, and translation, exhibit declining levels with aging [85]. Proteins like HuR and TIA-1 lose their ability to stabilize stress-response transcripts, weakening cellular repair mechanisms and exacerbating age-related damage [86]. Additionally, specific transcript variants like *Cdkn1a* variant 2, which encodes the P21CIP1/WAF1 protein, are a specific marker of aging and cellular senescence [87]. The elevated level of this isoform of *Cdkn1a* was observed in multiple tissues of aging mice, including the liver, adipose tissue, kidney, heart, and lung [88]. Post-transcriptional processes, including transcription elongation and alternative splicing, remain central to the cellular landscape of aging. In the human brain alone, over 1,174 exons exhibit differential expression with age, reflecting decreased alternative splicing across multiple tissues, including blood, skin, muscle, bone, thymus, spleen, and adipose tissue [81]. Accelerated transcription elongation increases splicing and raises the likelihood of erroneous splicing, which is detrimental to cellular functions.

One hallmark of aging is the acceleration of RNA polymerase II (Pol II) elongation speed across species and tissues [89]. This increased transcriptional speed disrupts the fidelity of RNA splicing, leading to reduced unspliced transcripts and elevated circular RNA formation [90,91]. Such changes alter isoform ratios and compromise protein activity, contributing to functional decline [92]. Genes involved in DNA repair and muscle homeostasis are particularly affected, with splicing errors resulting in aberrant isoforms that undergo nonsense-mediated decay [63]. It has been demonstrated that aging alters the splice variants of genes such as ESRRG and TET2 in skeletal muscle, which are essential for type-1 muscle fiber development and myogenic differentiation [92]. Aging also changes the splice variants of genes, which may cause muscle damage [92]. It has been found that lifespan-extending interventions, such as dietary restriction and reduced insulin-IGF signaling, can reverse those transcriptional and splicing alterations in skeletal muscle [93].

Aging also activates retrotransposable elements, increasing genomic instability as these mobile genetic elements generate cDNAs through reverse transcription [19]. This activity can lead to genomic reinsertion and DNA damage, induction of new mRNA variant expression, and exacerbation of cellular dysfunction. Interestingly, lifespan-extending interventions like caloric

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restriction have been shown to suppress retrotransposon activity in aged mice, highlighting the connection between retrotransposon regulation and longevity [94,95]. Declines in RNA quality control mechanisms further compromise mRNA integrity during aging [96]. In organisms like *C. elegans*, age-associated reductions in mannosyltransferase ALGN-2 impair nonsense-mediated mRNA decay, allowing harmful splicing errors to accumulate. These breakdowns contribute to cellular dysfunction across aging tissues [97].

3. mRNA Isoforms in Disease Conditions

mRNA isoforms, arising from alternative TSS, alternative splicing, RNA editing, and other post-transcriptional modifications, play a crucial role in systemic diseases by diversifying protein functions and altering cellular processes [98]. These isoforms enable fine-tuned regulation of gene expression, but their dysregulation can disrupt physiological balance and drive disease progression across multiple organs and systems [99]. The generation of mRNA isoforms plays a multifaceted role in disease conditions. Some mRNA isoforms are formed in the host cells in response to the disease, while abnormal mRNA isoforms can be responsible for the disease pathogenesis. For simple understanding, we have described these mRNA isoforms according to the types of human diseases. We have grouped the diseases into infectious and non-infectious diseases. Non-infectious diseases are further sub-grouped into chronic systemic diseases, autoimmune diseases, metabolic diseases, and tumors.

3.1. mRNA Isoforms in Infectious Diseases

mRNA isoforms play a key role in infectious diseases, influencing host-pathogen interactions, immune responses, and disease progression. Interaction between hosts and pathogens modulates cellular mechanisms, leading to alternative splicing and expression of transcript variants in both [100]. This dynamic interplay and expression of mRNA isoforms may result in the inhibition or promotion of the disease. Thus, exploring the transcript variants may help in understanding the molecular mechanisms underlying infectious diseases.

In viral infections, altered splicing often benefits the pathogen by enhancing the replication and survival of the virus. For example, Epstein-Barr virus (EBV) interferes with the host splicing machinery to skip exon 11 in the *MPPE1* gene, expressing an *MPPE1* isoform that contributes to EBV-related tumorigenesis [101]. Similarly, HIV-1 infection alters splicing in host genes like *CCNT1* and *RUNX1* [102,103]. Reduced expression of *RUNX1b* and *RUNX1c* transcript isoforms that usually inhibit viral replication increase viral titer [104,105]. HIV also promotes exon 7 skipping in *CCNT1*, suppressing transcriptional activation and maintaining latency in infected CD4+ T cells [102,103]. The Hepatitis B virus (HBV) is known to produce a spliced viral RNA isoform, *SP1* RNA, which dampens inflammatory responses in the host to evade immune detection and establish a chronic infection [106,107].

Bacterial infections exploit alternative splicing to manipulate host defenses. Host response to infections also changes the expression of mRNA isoforms to augment immune functions [108]. *Mycobacterium tuberculosis* (MTB) promotes exon skipping in the RAB8B gene to produce truncated isoforms that impair macrophage autophagy, allowing intracellular bacterial survival [109]. MTB further alters the splicing of the *IL-12Rβ* mRNA transcript to generate a shorter isoform (IL-12Rβ1ΔTM) that enhances dendritic cell migration and MTB-specific T cell activation, promoting bacterial survival while modulating immune responses [110,111]. Other bacteria, like *Listeria monocytogenes*, influence the host splicing machinery through toxins like Listeriolysin O [112]. This toxin induces the alternative splicing of the cold-inducible RNA-binding protein (CIRBP), resulting in two isoforms with opposing actions. While CIRBP-201 reduces intracellular bacterial load by promoting immune responses, CIRBP-210 facilitates bacterial survival by supporting stress-related pathways [112,113]. Similarly, during *Salmonella typhi* infections, alternative splicing of interleukin-15 (IL-15) mRNA produces isoforms that either enhance interferon-gamma production for effective bacterial control or weaken immune responses to allow bacterial proliferation [114].These adaptive

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changes highlight the dual role of mRNA isoforms in infection-either bolstering host defenses or inadvertently supporting pathogen survival [115].

Parasitic infections also leverage mRNA isoform regulation to disrupt the host cellular functions. In trypanosomiasis, skipping exon 15 of the HDAC7 gene generates isoforms that inhibit host cell cycle pathways, promoting parasite survival and persistence [116,117].

3.2. mRNA Isoforms in Non-Infectious Diseases

mRNA isoforms also play a critical role in the pathogenesis and prognosis of non-infectious diseases. While pathogenic transcript variants cause certain diseases, others are associated with the progression of diseases and the response to conventional or targeted therapies. In the following section, we have discussed the role of mRNA transcript variants in autoimmune diseases, systemic and metabolic diseases, and tumors.

3.2.1. mRNA Isoforms in Autoimmune Diseases

Studies have shown that mRNA isoforms play a significant role in autoimmune diseases. Alternative protein isoforms profoundly impact immune signaling, antigen presentation, and inflammatory responses [118,119]. This process can also disrupt immune tolerance and contribute to tissue damage in autoimmune diseases [118,119]. In the following section, we discuss several autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome, multiple sclerosis (MS), Graves' disease, inflammatory bowel diseases (IBD), and type 1 diabetes mellitus (T1DM).

In SLE, alternative splicing is dysregulated, particularly affecting the genes crucial for immune regulation. Genome-wide studies have revealed widespread intron retention and exon skipping in immune-related genes in SLE patients, underscoring the impact of splicing dysregulation [120]. Splicing changes in *IRF5* transcript that encode transcription factors involved in interferon signaling generate isoforms that amplify pro-inflammatory cytokine production, exacerbating the chronic inflammation in SLE [121,122]. Similarly, variants in the CTLA4 gene produce isoforms that modulate T-cell activation differently, increasing susceptibility to SLE [123,124]. The exact mechanism can also play a causative role in other autoimmune conditions, such as RA and T1DM [123,124]. In RA, the alternative splicing of the CD44 gene, which encodes a cell surface receptor involved in leukocyte migration and inflammation, results in the production of multiple isoforms [125,126]. Altered splicing of the *TNFRSF1B* transcript, which encodes the tumor necrosis factor receptor 2 (TNFR2) protein, a soluble isoform of the receptor that modulates TNF signaling and perpetuates chronic inflammation in RA [127]. While increased expression of *IRAK1* isoforms enhances the interferon signaling in SLE, an elevated level of non-functional *SIGLEC10* isoforms impairs the anti-inflammatory activity in RA (128-130). Another autoimmune disease, Sjögren's disease, can occur by itself or alongside other autoimmune diseases like SLE and RA. Studies have shown that transcript variants of several genes involved in immunity are dysregulated. A genetic variant (rs10774671) alters the splicing of *OAS1*, resulting in the production of alternative isoforms, such as p42, p48, and p44, rather than the p46 isoform [131]. The isoforms impair responsiveness to IFN stimulation and contribute to the production of autoantibodies, leading to chronic inflammation (132-134). Studies have shown that dysregulated isoforms in genes like *STAT4* and *IRF5* influence inflammation and fibrosis in another autoimmune disease known as scleroderma [135,136]. Increased expression of *NBPF* family transcripts in dermal fibroblasts drives abnormal tissue remodeling, while splicing alterations in TGF-β signaling pathways further amplify fibrotic processes [137].

In MS, dysregulation of RNA splicing leads to the expression of autoantigens from the genes that generally maintain myelination of axons [138]. These alterations exacerbate immune-mediated damage to the central nervous system, contributing to the pathogenesis of MS [139]. Splicing changes in the *MOG* gene generate isoforms targeted by autoantibodies, while defects in the *PLP* gene contribute to demyelination and neurodegeneration [140]. Myasthenia gravis (MG) is a chronic autoimmune disorder that causes muscle weakness and fatigue. In MG, alternative splicing impairs

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neuromuscular signaling and the host immune system mistakenly attacks the connection between the nerves and muscles. It has been shown that the isoforms of the *CHRNA1* gene disrupt acetylcholine receptor assembly, thereby weakening neuromuscular communication and leading to muscle weakness [141].

Graves' disease, an autoimmune thyroid disease, highlights the role of alternative splicing in thyroid dysfunction. The TSH receptor (*TSHR*) gene produces truncated isoforms, such as ST4 and ST5, that impair central tolerance by allowing autoreactive T cells to target the TSHR [142,143]. The production of autoantibodies against the TSHR affects TSH-signaling and leads to hyperthyroidism. Altered splicing of other thyroid function-related genes, like thyroid peroxidase (*TPO*), further enhances autoantigenicity [142,144].

Pathogenesis of inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are also impacted by alternative isoform generation [145]. Splicing of the *STAT3* gene produces isoforms with distinct immune regulatory functions [146]. Dysregulated splicing of *MLCK* generates variants, such as MLCK2, which increases intestinal permeability and promotes bacterial invasion, thereby worsening inflammation [147,148]. Splice variants of the glycoprotein 2 (*GP2*) gene also function as autoantigens, targeted by autoantibodies, which enhance bacterial adhesion to the intestinal epithelium and drive disease progression in IBD [149]. In Crohn's disease, altered splicing of *NOD2* transcript impairs bacterial sensing. *LACC1 t*ranscript variants expressed from alternative promoters inhibit the differentiation of regulatory T cells, thereby increasing inflammation. Isoforms of IL-23R alter the IL-23/IL-17 axis, a critical pathway in Crohn's disease pathogenesis (150-152). In ulcerative colitis, splicing changes in genes like *PTPRS* produce isoforms that alter receptor function, disrupting immune signaling and mucosal repair [153]. For instance, the loss of exon 9 in *PTPRS* affects ligand recognition and dimerization, contributing to colitis progression [154].

In T1DM, splicing changes in autoantigen-encoding genes play a pivotal role [155]. The IA-2 gene produces alternatively spliced isoforms that alter its immunogenicity, influencing how autoreactive T cells target pancreatic beta cells. As mentioned above, alternative splicing of CTLA4 generates soluble isoforms that disrupt T-cell regulation, exacerbating the autoimmune attack on insulin-producing cells and accelerating disease progression (156-158).

Sarcoidosis is a chronic inflammatory disease that affects the lungs, skin, or lymph nodes. It is not a typical autoimmune disease, but its pathogenesis shares similarities with those of other autoimmune diseases. Sarcoidosis has mRNA isoforms that contribute to inflammation and the formation of granulomas. Aberrant expression of *THE1B* fusion isoforms correlates with disease activity, whereas splice variants such as *SIRPB1-SIRPD* activate NF-κB pathways in granuloma-associated macrophages. Isoforms of IGF-1 contribute to fibrotic changes that worsen the progression of sarcoidosis [159,160].

3.2.2. mRNA Isoforms in Chronic Systemic Diseases

mRNA isoforms, produced through alternative pre-mRNA processing and the use of alternative TSS, play a significant role in various disease conditions [161]. Disruption of the normal isoform versus pathogenic variants balance contributes to pathological mechanisms [161]. Among the systemic diseases, neurological disorders are the most common chronic diseases that result from pathogenic transcript variants. Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD) are deeply intertwined with the dysregulation of mRNA isoform generation.

In Alzheimer's disease, changes in the ratio of amyloid precursor protein (APP) isoforms are linked to plaque formation and neuronal damage [162]. Splicing changes in MAPT generate tau isoforms that aggregate into neurofibrillary tangles, a hallmark of AD [162,163]. A recent study has identified read-through transcripts, such as *TOMM40-APOE*, at Alzheimer's susceptibility loci, which are linked to disease risk. Similarly, neuropsychiatric disorders involve novel mRNA isoforms of *ATG13* and *GATAD2A* genes, which alter the protein domains and play a pathogenic role [164]. In

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PD, variants of the *SNCA* gene alter the alpha-synuclein expression, leading to toxic Lewy body formation [165]. It has been found that mutations in the *TARDBP* gene impair splicing regulation, leading to the production of abnormal protein isoforms that drive motor neuron degeneration in ALS [166,167]. ALS and FTD involve mutations in genes like *C9ORF72*, which produce RNA sites that disrupt splicing regulation. These mutations lead to mis-spliced TDP-43 aggregates in the cytoplasm, damaging neurons [168,169]. Similarly, in Huntington's disease-like 2 (HDL2), splicing defects in the *JPH3* gene lead to toxic RNA gain-of-function effects that disrupt ion channel activity [170,171]. Retinal degeneration further underscores the significance of isoform-specific functions of the CRB1 gene, which is essential for photoreceptor cell integrity, and the loss of CRB1 function results in increased cell death [172,173]. Retinitis pigmentosa also exemplifies the consequences of splicing-related mutations; alterations in genes such as PRPF31 and PRPF8 impair mRNA processing, leading to photoreceptor degeneration and progressive vision loss [174,175]. Beyond neurological conditions, isoform dysregulation impacts neuromuscular diseases such as muscular dystrophy. Mutations in the *DMD* gene disrupt dystrophin isoforms critical for maintaining muscle integrity and brain function, with the loss of neuronal isoforms, such as *DP427*, leading to cognitive impairments alongside muscle degeneration [129,176]. Fibromyalgia shows how mRNA isoform dysregulation affects systemic pain sensitivity. Isoforms of the SCN9A sodium channel gene influence nerve excitability, while serotonin receptor polymorphisms, such as 5-HT2A, are linked to widespread chronic pain in fibromyalgia [177,178].

Cardiovascular diseases are another domain where mRNA isoforms play a pivotal role. In heart failure, splicing changes in sarcomeric genes like *TTN* produce isoforms that affect myocardial stiffness and contraction [179]. Similarly, alternative splicing of *TNNT2* and *MYH7* alters proteins essential for cardiac muscle function. During myocardial infarction, spliced isoforms promote cell survival and repair mechanisms, while circular RNAs derived from spliced transcripts regulate gene expression by acting as microRNA sponges [180]. In cardiac hypertrophy, splicing factors like RBM20 control isoform changes that adapt the heart's mechanical properties under stress [181,182]. Hypertension also highlights the role of mRNA isoforms in systemic diseases. Variants in genes like *KLF4* and *KLF5* affect vascular smooth muscle cell (VSMC) behavior, contributing to blood pressure regulation and vascular remodeling [183]. For instance, the *KLF5* variant rs9573096 promotes VSMC dysfunction, increasing hypertension risk, while elevated *KLF4* mRNA levels drive VSMC proliferation and worsen vascular injury [183,184].In atherosclerosis, transcript length variations in genes like *MIA3* reduce VSMC proliferation and impair plaque stability, increasing cardiovascular risk [185,186].

Chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) also exhibit significant mRNA isoform dysregulation [187]. In asthma, alternative splicing of the *ADAM33* gene generates variants that lack catalytic domains required for metalloprotease activity, thereby affecting airway remodeling. Variants of *TGFBR1* and *SMAD3*, key regulators of TGF-β signaling, contribute to airway inflammation and fibrosis by altering immune cell regulation [188,189]. COPD is characterized by frequent exon skipping and reduced intron retention in severe cases, resulting in protein-coding isoforms that impact lung tissue remodeling and inflammation [190]. For instance, spliced variants of the SERPINA1 gene reduce alpha-1 antitrypsin levels, leading to tissue damage and emphysema [191,192].

Other systemic diseases, such as osteoporosis and cataracts, are also influenced by mRNA isoform dysregulation. Cataracts arise from abnormal protein aggregation caused by mutations in the lens-specific *CRYBB2* gene [193]. The variant *CRYBB2*:c.62T>A affects protein folding and induces cellular stress, leading to lens opacity [194,195]. In osteoporosis, variants in genes such as LRP5 impair WNT signaling pathways, which are critical for osteoblast activity and bone formation [196].

3.2.3. mRNA Isoforms in Tumors

Numerous studies have demonstrated that mRNA isoforms play a crucial role in tumor biology by diversifying the transcriptome. Dysregulation of the mechanisms for expressing normal mRNA

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isoforms may result in the expression of growth-promoting transcript variants. Differential expression of such mRNA isoforms may be characteristic of benign from malignant tumors [197]. It has been demonstrated that aberrant splicing is linked to tumor progression, metastasis, and resistance to chemotherapy or radiotherapy [198,199]. It has been reported that splicing events in BCl2L1 produce anti-apoptotic BCL-XL isoforms that protect cancer cells from programmed cell death [200]. Similarly, aberrant splicing of caspase genes generates isoforms that suppress apoptosis, ensuring tumor survival. These changes are often driven by dysregulated oncogenic signaling pathways such as RAS/RAF/ERK or PI3K/AKT/mTOR, which modify splicing factor activity at transcriptional or post-translational levels [201,202].

A tumor-specific variant of the epidermal growth factor receptor (*EGFR*) gene, *EGFRvIII*, is expressed in glioblastoma [203]. EGFRvIII alters signaling pathways to promote proliferation and resistance to targeted therapies. Notably absent in normal tissues, this isoform represents a promising therapeutic target due to its specificity for malignant cells [204,205]. In glioblastoma, RNA editing creates protein isoforms that evade immune responses while activating oncogenic pathways. These post-transcriptional modifications contribute to therapeutic resistance and tumor growth [206]. Similarly, in prostate cancer, the *TMPRSS2-ERG* fusion transcript exemplifies the oncogenic potential of an mRNA isoform [207]. This fusion is induced by androgen signaling and leads to the expression of the ERG proto-oncogene, which enhances tumor invasion and angiogenesis, contributing to aggressive disease phenotypes [208,209]. In thyroid cancer, microarray analyses have identified transcriptomic differences between subtypes, with differential expression of isoforms in malignant cells [210]. Similar findings in breast cancer also revealed overexpression of tumor-specific isoforms that correlate with enhanced proliferation and resistance to chemotherapeutics [211]. Long-read sequencing has uncovered thousands of novel isoforms in breast cancer, many of which affect protein function and localization [29]. Alternative splicing also plays an important role in metabolic reprogramming and immune evasion in cancer [212].

Variations in UTRs influence mRNA stability and translation by altering interactions with microRNAs or various RNA-binding proteins (RBPs). These changes can upregulate oncogenes or downregulate tumor suppressors, contributing to cancer progression [213]. For example, germline variants in the 3′ UTR of the *CDKN2A* gene, such as 500C>G and 540C>T, have been associated with increased melanoma risk and poorer prognosis by disrupting RBP binding and destabilizing mRNAs [214].

4. Translational Relevance of mRNA Isoforms

Advances in RNA sequencing have further enhanced our ability to detect these variants, revealing previously unannotated transcripts that refine our understanding of gene regulation and disease mechanisms [29,215]. It has been demonstrated that identifying isoforms enables the knowledge of how genes are expressed under physiological conditions and their contribution to disease [216]. The mRNA isoforms expressed from these genes have diverse roles in development, organogenesis, aging, and disease pathogenesis, making them critical targets for clinical applications. Recent studies on mRNA isoforms have revolutionized translational research by uncovering the intricate regulatory mechanisms underlying disease pathogenesis [217]. For example, germline mutations 3′ UTR of CDKN2A disrupt RBP binding, destabilizing mRNA and increasing the risk of melanoma [218].

In diagnostics, transcript variants have proven invaluable for distinguishing disease states. For example, specific isoforms, such as *CLU2*, in thyroid cancer can differentiate between malignant and benign tumors, enabling more accurate diagnoses and guiding preoperative decisions [197]. Transcriptomic profiling in breast cancer has also identified isoforms overexpressed in malignant cells, providing biomarkers for predicting disease progression and developing therapeutic strategies [219].

Therapeutically, targeting mRNA transcript variants has emerged as a promising approach for addressing genetic disorders and certain types of cancer. Splice-site-directed oligonucleotides and

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antisense therapies are developed to correct aberrant splicing and inhibit expression of pathogenic transcripts [220]. Recently, spliceosome modulators targeting *SF3B1* mutations have been utilized in the treatment of hematological malignancies and solid tumors. FDA-approved therapies utilize these technologies to modulate RNA stability and translation, offering precision medicine (221-223). Large-scale transcript variants, identified through advanced techniques like full-length ribosome–nascent chain complex sequencing, have revealed novel neoepitopes derived from unannotated proteins [224]. These neoepitopes expand the repertoire of potential targets for personalized cancer vaccines, offering hope for more effective treatments across diverse tumor types [225,226]. Beyond diagnostics and therapeutics, transcript variants are reshaping the landscape of immunotherapy.

Transcript variants also play a crucial role in pharmacogenomics, influencing drug metabolism and response. Variations in UTRs can alter mRNA stability and translation efficiency through interactions with microRNAs or RBPs, impacting how the body processes drugs [227]. Variations in drug-metabolizing enzymes like CYP3A4 affect statin dose requirements, underscoring the importance of transcript variant analysis in optimizing therapeutic outcomes [228].

5. Conclusions

Multiple mRNA isoforms can be expressed from a single gene, which varies in their 5′ end, 3′ end, or internal coding sequences. Thus, a single gene can express multiple proteins with diverse functional domains. The transcript variants may be expressed in varying quantities in different cell types or within the same cell type under different physiological or pathological conditions. The mRNA isoforms and the transcript variants can play diverse roles in cell differentiation, organogenesis, and aging processes. Recent studies suggest that the expression of alternative transcript variants is linked to disease pathogenesis and can impact disease progression or response to therapeutics. This article discusses published reports on the physiological and pathological roles of mRNA transcript variants. These studies emphasize the importance of examining mRNA transcript variants rather than focusing solely on a single mRNA isoform expressed from a gene. Nowadays, long-read RNA sequencing can accurately identify mRNA isoforms. However, future developments in proteomic techniques will be necessary to identify the corresponding protein isoforms.

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