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Blocking Translation of Oncogenic mRNA

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Abstract

Double-stranded RNA-mediated interference (RNAi), antisense oligonucleotides (ASO), and ribozymes have excellent specificity to their target oncogenic mRNA. They also seem to show great promise when it comes to treating cancer. The problem is that RNAi, ASO, and ribozymes have poor stability and are constantly being degraded by nucleases. Researchers have made some efforts to increase antisense oligonucleotides' stability by creating phosphorimidate and Phosphorothioate. Currently, ribozymes, antisense oligonucleotides, and (RNAi) are the three main methods used to target RNA. These methods are currently undergoing clinical trials for the purpose of focusing on specific RNAs involved in disorders like cancer and neurodegeneration. In fact, ASOs that target amyotrophic lateral sclerosis and spinal muscular atrophy have produced promising results in clinical trials. The formation of chemical alterations that boost affinity and selectivity while reducing noxiousness owing to off-target impacts are two benefits of ASOs. Another benefit is increased affinity. With a focus on RNAi and ASOs, this review illustrated the main therapeutic strategies of RNA therapy now in use.

Keywords

Antisense Oligonucleotides, Ribozymes, Phosphorothioate, Double-Stranded RNA-Mediated Interference, Nucleases

1. Introduction

After cardiovascular diseases, cancer causes most of the deaths all over the world [1] [2]. Hence, Cancer is one of the leading causes of death worldwide, and its treatment remains a major challenge for medical science. Over the years, researchers have developed different approaches to treating cancer, including surgery, radiation therapy, and chemotherapy. While these therapies have shown some success in treating cancer, they often come with significant side effects and limitations [3]. With advances in genomics, scientists have discovered new ways

to target specific genes and pathways that contribute to cancer growth and progression. RNA therapy is one such approach that has emerged as a promising tool for cancer treatment [4].

Cancer progresses cellular genes for the reproduction, differentiation and survival are mutated. Mutations maybe develop in both proto-oncogenes and suppressor genes. When these mutations in proto-oncogenes so they become hyperactive and hence known as oncogenes. Cell growth is inhibited by suppressor genes or the cell cycle and, when mutation occurs in these genes, growth and cell division is uncontrolled. These mutations affect DNA repair genes then cause unrepaired mutations in other genes [5]. Oncogenes are a set of genes that have the potential to convert normal cells into cancerous cells [6]. These genes can be mutated or expressed highly in cancerous cells. With the advancement in knowledge, science has solved many confusing questions by tackling basic and translational research on how certain oncogenic mutations are causing cancer [7]. In the past few years, with the rapid advancement in genomics, target-specific therapies have been used to treat a number of human cancers. Target-specific therapies showed better results than conventional chemotherapy, during clinical trials on cancer patients [8] [9] [10].

Researchers have discovered many struggles in inhibiting malignant transformation at the gene level to treat cancer. These therapies fall within four broad groups:

1) Gene Interference;

2) Gene Replacement;

3) Immunopotentiation;

4) Insertion of Suicide Genes.

Gene interference encompasses RNA therapeutics. RNA therapeutic refers to double-stranded RNA-mediated interference (RNAi), antisense oligonucleotides (ASO), and ribozymes. Clinical trials with ASOs that target amyotrophic lateral sclerosis and spinal muscular atrophy have yielded promising results. The invention of chemical alterations that boost specificity and affinity simultaneously reducing toxicity owing to off-target consequences are two benefits of ASOs. Another benefit is increased affinity. Researchers use double-stranded RNA-mediated interference (RNAi), antisense oligonucleotides (ASO), or ribozymes to inhibit the translation of oncogenic mRNA.

RNA-mediated interference (RNAi) is a process in which small double-stranded RNA molecules, called small interfering RNAs (siRNAs), bind to and cleave complementary target messenger RNA (mRNA) molecules, resulting in inhibition of protein translation [11]. RNAi activates ribonucleases and cooperates with other enzymes to degrade the fragmented oncogenic RNA. Antisense oligonucleotides (ASOs) are short single-stranded DNA or RNA molecules that are designed to bind to complementary mRNA sequences, leading to the degradation of the mRNA or preventing its translation into protein [12]. Antisense oligonucleotides anneal with the oncogenic mRNA's bases and prevent translation via "steric hindrance, splicing alterations, initiation of target degradation, or

other events" [13]. Ribozymes are RNA molecules that possess enzymatic activity and can catalyze the cleavage of specific RNA molecules. While, Hammerhead ribozymes are a type of ribobozymes that can cleave specific oncogenic mRNAs [14]. Hammerhead ribozymes break the bcr-abl mRNA at the breakpoint. The breakpoint is the point separating the bcr portion from the abl part of the mRNA.

RNA therapy is revolutionary to cancer therapy as it targets the oncogenic mRNA. Treatment that targets the oncogenic proteins is generally toxic to non-malignant cells. The use of RNA therapy allows researchers to prevent the production of an oncogenic protein with excellent specificity and without significant toxicity. There are drawbacks to RNA medicines. Due to RNAs being unstable and the kidneys excreting RNAs, researchers have difficulty transfecting target tissue. There are also growing concerns with RNA base therapies inhibiting unintended mRNA.

This review aims to provide an overview of the emerging field of RNA therapy (gene interference) for cancer treatment. RNA therapy involves using small RNA molecules to target and inhibit the expression of specific genes involved in cancer development and progression. This approach has the potential to overcome some of the limitations of traditional cancer therapies, such as chemotherapy and radiation, which can have significant side effects and may not be effective against all types of cancer. Moreover, the advantages and challenges associated with RNA therapy and some recent advancements in the field will be analyzed.

2. RNA Interference (RNAi) Pathway

The RNAi pathway can be represented simply as two stages relating to the ribonuclease enzyme. The trigger RNA is transformed into a short, interfere with RNA in the first stage by the RNase II enzymes Dicer and Drosha (siRNA). In the following phase, siRNAs are exposed onto the effector complex of the RNA-induced silencing complex (RISC). The siRNA is unraveled during RISC formation, and the single-stranded RNA then combines with the mRNA receptor. The specific mRNA is nucleolytically degraded by the RNase H enzyme Argonaute, silencing the gene. The mRNA is not sliced if the siRNA/mRNA duplex has incongruities. Relatively, translational inhibition is the reason of gene silencing (**Figure 1**) [15].

Currently, Yeo's group has observed the role of various binding proteins associated with RNA through CRISPR-Cas9 transmission in cancer and discovered about 57 RNA binding proteins with important function in promoting MYC-driven oncogenic pathways (Einstein *et al*., 2021). The studies show some therapeutic applications of these RNA binding proteins by investigating the critical role of YTHDF2 protein in regulation of transcription of MYC-directed breast cancer [16]. Other than using target protein-coding elements for treatment of cancer, there are some non-coding elements such as miRNAs that have crucial roles in the tumor microenvironment regulation. These non-coding elements

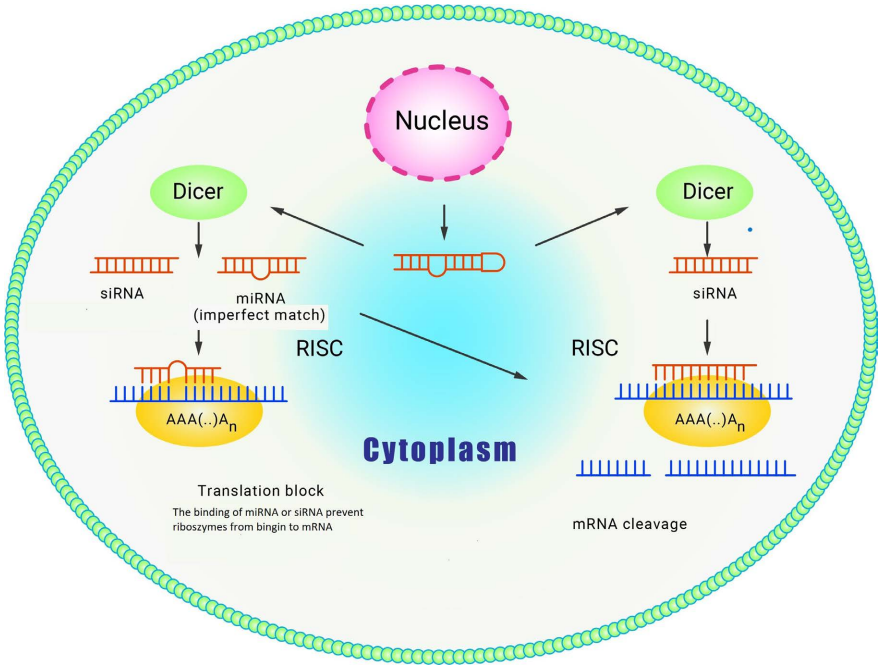

Figure 1. Mechanism for the RNAi pathway.

control gene expression at chromatin or post-transcriptional level, which regulates the oncogenic physiology and other transformation processes [17]. A new era of RNA therapeutics is attracting researchers. RNA therapeutics is a better option in treating cancer by adjusting the expression of various marker proteins other than conventional methods such as use of molecular drugs, inhibitors, antibodies etc. As compared to molecular or proteins based cancerous treating drugs, RNA therapeutics can easily be designed. Nusinersen, an FDA approved antisense oligonucleotide (ASO), is being used for treatment of muscular atrophy in children [17] [18]. Surprisingly, immunoregulatory effects observed with the use of RNA molecules like small interfering RNA [siRNA], microRNA [miRNA], and messenger RNA [mRNA], showed potential effects for cancer chemotherapy. RNA-based therapy stimulates both innate and adaptive immunity by regulating or silencing immunity-related genes and modulating cytokine expression [2] [19].

Researchers doing research with plants discovered RNAi. However, Researchers did not understand RNAi's mechanism of action only Fire and Mello's landmark explained about this. The landmark article explained that double-Stranded RNA (dsRNA) inhibits mRNA in *Caenorhabditis elegans*.

The following is the function of RNAi:

- maintenance of germline integrity;
- prevent the conversion of transposons into mRNA;
- prevent the conversion of mRNA into protein [20].

Intracellular or extracellular dsRNA can activate the innate immune response. Due to the human body's inability to degrade dsRNA, evolution has evolved another way of purging the dsRNA by triggering interferon. Besides interferon

increasing adjacent cells' resistance to viral infection, researchers discovered that small RNAs utilize RNAi machinery to silence oncogenic RNA. The three-class of small RNA that work with RNAi machinery to silence target mRNA are:

1) Piwi-interacting RNAs (piRNAs);
2) MicroRNA (miRNAs);
3) Small interfering RNA (siRNAs).

2.1. Piwi-Interacting RNAs (piRNAs)

piRNAs are a class of small non-coding RNAs that are primarily found in germ cells, where they play a crucial role in protecting the genome from the deleterious effects of transposable elements (TEs) and maintaining genome stability [21]. TEs are repetitive DNA sequences that can move around and disrupt the genome, leading to mutations, chromosomal rearrangements, and genomic instability. Germ cells are particularly vulnerable to TEs because they undergo extensive DNA replication and meiotic recombination, which can create new mutations and rearrangements that can be passed on to the next generation.

piRNAs are generated by a distinct biogenesis pathway that differs from the pathways for miRNAs and siRNAs In mammals, piRNAs are generated from several different types of genomic loci, including piRNA clusters, transposon-free regions, and genic regions. The specific piRNA-producing loci and piRNA populations are regulated by a complex interplay between epigenetic factors, transcription factors, and post-transcriptional processing [22].

piRNAs, which have a length of 21 - 35 nucleotides, suppress transposable elements, control gene appearance, and prevent viral contamination. The piRNAs direct the PIWI proteins to fragment the target RNA, encourage the construction of heterochromatin, and methylate DNA. The piRNA pathway's framework supports it to control conserved host genes in addition to providing adaptive, sequence-sbased resistance to transposons and viruses that are continually developing. While somatic piRNA activities have been repressed, recovered, or misplaced then during evolution, piRNAs silence jumping gene in the germ lines of the majority of mammals. The majority of piRNA processing proteins are also highly conserved, although the methods used by various animals to generate piRNA precursor transcripts are strikingly diverse [23].

2.2. siRNAs

Long dsRNAs are precursors for siRNA. Dicer binds to the long dsRNAs and cuts them into short double-strand RNA. RNA-induced silencing complex (RISC) and endonuclease Argonaute 2 then attach to the small piece of Double-Stranded RNA and select a single-stranded guide RNA (antisense RNA). The guide RNA then binds to the target RNA by Watson and Crick's model, allowing the AGO2 to cleave the target mRNA. Exonuclease then degrades all cleaved mRNAs.

Patisiran (Onpattro) is an FDA-approved RNA-based therapy for hereditary transthyretin-mediated amyloidosis (hATTR). Patisiran is a double-stranded

siRNA that targets the mutant transthyretin mRNA and reduces the levels of the mutant protein that accumulates in tissues and causes the disease [24].

Inclisiran is an RNA-based therapy developed by Novartis that targets PCSK9, a protein that regulates cholesterol metabolism. Inclisiran is a double-stranded siRNA that is conjugated to a lipid nanoparticle and delivered subcutaneously. Inclisiran has shown promising results in clinical trials for the treatment of hypercholesterolemia [25].

QR-421a is an RNA-based therapy being developed by ProQR Therapeutics for the treatment of Usher syndrome type 2A, a genetic disorder that causes deafness and blindness. QR-421a is an antisense oligonucleotide that binds to a specific exon in the USH2A gene and promotes exon skipping to restore the production of functional protein. QR-421a is delivered via intravitreal injection into the eye [26].

The challenges that researchers face with designing siRNA RNA therapy are the RNA binding to unintended mRNA, the ability for the siRNA RNA therapy to produce its intended result, delivering the siRNA RNA therapy to its intended target, and the initiation of the immune system. The siRNA can bind imperfectly to unintended mRNA, or the siRNA can enter the miRNA system, increasing the tolerance for imperfect binding of unintended mRNA. siRNAs accurately inhibit mRNA translation with 100% efficiency [27]. The guide RNA not having accessibility to the target mRNA's complementary sequence can reduce siRNA's efficiency. Non thermodynamically stable siRNA, when integrated into the RISC complex, can reduce siRNA's efficiency; modification of siRNA to be resistant to nuclease can also reduce siRNA's efficiency.

To address this challenge, various delivery strategies have been developed, including the use of lipid nanoparticles, viral vectors, and cell-penetrating peptides. These delivery vehicles can protect the RNA molecules from degradation and facilitate their entry into cells. However, each delivery strategy has its own advantages and limitations, and optimizing the delivery system for each RNA-based therapy is a complex and time-consuming process [28].

Another issue that affects the transfer of siRNA to the target tissue is that the kidney filters out siRNA and nucleases within the blood, degrading the siRNA. Researchers have combated nucleases degrading siRNA by converting siRNA into phosphorothioate and phosphoramidate. The problem with creating these compounds is that chemical modification of siRNA can hamper the efficacy of siRNA by triggering the innate immune system response and inducing proinflammatory cytokines. Researchers have discovered chemical modification techniques that prevent siRNA from triggering said immune response and proinflammatory cytokines. However, researchers must be cautious in such modification techniques because of steric hindrance. Steric restriction can prevent the double-stranded-cleaved-siRNA from integrating into the RISC complex [29].

2.3. miRNAs

Short non-coding RNAs having 18 - 22 nucleotides in length that modulate the

degeneration and stability of mRNA utilizing natural RNAi machinery called mi RNAs. The processes used by miRNAs and siRNAs to modulate the synthesis of target transcripts are same and both use an RNA-induced silencing complex to target any transcript complex. siRNAs accurately degenerate mRNA translation with 100% efficiency in comparison to miRNAs, whose complementarity with the sequences is incomplete to do gene silencing via slicer-independent pathways [27]. miRNAs have impact on immune cell functions and via a molecular process command immune cells to attack on cancer [30]. miRNAs have role in cancer immune supervision [31].

MiR-155 is highly expressed in activated immune cells and plays a critical role in regulating the function of various immune cells, including T cells, B cells, dendritic cells, and macrophages. MiR-155 has been shown to promote the differentiation and activation of Th1 and Th17 cells, as well as enhance the antibody response of B cells. However, excessive expression of miR-155 can lead to chronic inflammation and autoimmune diseases. Several ASOs targeting miR-155 are currently being tested in preclinical and clinical studies for the treatment of various autoimmune diseases, including multiple sclerosis, lupus, and rheumatoid arthritis [32].

Likewise, MiR-146a is a negative regulator of the immune response and is involved in maintaining immune homeostasis. MiR-146a has been shown to regulate the function of various immune cells, including macrophages, dendritic cells, and T cells. Overexpression of miR-146a has been associated with the suppression of pro-inflammatory cytokines and the promotion of anti-inflammatory cytokines [33]. Several studies have demonstrated the potential therapeutic benefits of targeting miR-146a in various inflammatory diseases, including rheumatoid arthritis [34], systemic lupus erythematosus, and inflammatory bowel disease [35].

Alizadeh *et al.*, (2029) also studied MiR-29b miRNA which plays a critical role in regulating the function of T cells and is involved in the pathogenesis of several autoimmune diseases. MiR-29b has been shown to promote the differentiation of Th17 cells, which are involved in the development of various autoimmune diseases [36].

Various studies have shown that tumor-suppressing miRNAs have regulated antitumor immune reaction within the tumor microenvironment (TME) by controlling ICPs such as PD-1, PD-L1, and CTLA-4 [37]. Given that numerous genes are controlled by miRNAs, targeting miRNAs with ASOs seems to be a more popular and efficient strategy. In reality, studies in mice, and people have shown that ASOs that target miRNAs are effective and harmless [38].

MiRNA has a similar mechanism of action as siRNA when bound to AGO2-RISC complex. The difference is that miRNA typically can regulate more target mRNA than siRNA and another difference is how miRNA's single stranded guide RNA is formed. The genetic precursor of miRNAs can be naturally found or be inserted into the genome by a non-competent viral vector. Polymerase II is responsible for transcribing the genetic information of miRNA to primary miR-

NA. Once transcribed the primary miRNA fold on itself and forms a hairpin structure. Drosha then cleave primary miRNA into pre-miRNA. At this point the pre-miRNA is about 100 nucleotides long with a hairpin loop and partially double stranded. Exportin 5 then transport pre-miRNA to the cytoplasm. Drosha then cleave the hairpin loop from the pre-miRNA [39].

RNAse III enzyme Dicer cleave miRNA from the hairpin loop. The miRNA then binds to the RISC complex by binding to AGO2. AGO2 chooses the guide strand and discard the passenger strand. Seven bases near the 5 prime end forms the seed sequence. The seed sequence is responsible for forming imitating Watson-Crick base pairing with the target mRNA. The guide RNA binds to the target mRNA and AGO2 cleave the target mRNA. Exonuclease then degrades all cleaved mRNAs. The same challenges face siRNAs also face miRNAs.

3. mRNA Untranslated Regions in Cancer

The proteome diversity depends on post-transcriptional events, which are responsible for about 65% of the diversity in gene regulation. The mRNA domains 5' and 3' UTRs play a crucial role in post-transcriptional parameter of gene look. The 5' and 3'UTRs contain a wide range of regulatory different components in pre-mRNA processing, mRNA integrity, and translation activation as often transcribed but infrequently translated regions that are often transcribed but infrequently translated [40]. The 5' and 3' UTRs are also the 5' and 3' UTRs are developing Cis-Regulatory Regulation of Cancer Gene Expression. The primary mechanism by which the 5' and 3' UTRs function is a dynamic interaction among RNA-binding proteins (RBPs) or short RNA trans-acting elements and nucleotide and structural motifs generally known as cis-regulatory factors. They work together to control the metabolism and synthesis of particular mRNAs, which in turn shape the cellular proteome. Furthermore, by promoting the expression of carcinogenic genes, dysregulation of cis-regulatory components can promote the development of cancer [17].

3.1. 5' UTR Upstream Open Reading Frames

A special 5' UTR component called the upstream open reading frame (uORF) has the power to control how some transcripts' translation is initiated. It is an ORF that can be translated and has a start codon that comes before the principal ORF initiate codon. While there is disagreement over whether uORFs are translated into useful peptides, it has been demonstrated that their existence on mRNA transcripts affects how the primary subsequent ORF is expressed [41].

Researchers have found that tumor suppressor gene (TP53) mRNA contains two uORFs in its 5' UTR, which can inhibit the translation of the downstream coding region of TP53. However, when cells are exposed to stress, such as DNA damage, the translation of TP53 is increased through the regulation of uORF1 and uORF2. This mechanism ensured that TP53 is only expressed when necessary, preventing the accumulation of TP53 protein in non-stressed cells [42].

Moreover, tRNA modification can also play a critical role in cancer development. N7-methylguanosine (m7G) modification of tRNA can be altered in cancer cells. Specifically, m7G modification was found to be decreased in lung cancer cells, which led to the misreading of codons during translation and increased protein diversity. This alteration was also found to contribute to the formation of tumors in vivo. Hence, targeting tRNA modification could be a potential therapeutic strategy for cancer treatment [43].

3.2. N7-Methylguanosine tRNA Alteration Increases Oncogenic mRNA Translation

In m7G-tRNA-decoded intron processes, m7G tRNA modification preferentially affects the translation of carcinogenic transcripts, involving cell division process and genes associated with epidermal growth factor receptor (EGFR) pathway. Furthermore, we show how Mettl1-mediated m7G tRNA alteration is essential for ICC carcinogenesis and development in vivo utilizing amplification and deletion mice models [44]. Our research reveals the crucial physiological role and procedure that METTL1-mediated m7G tRNA alteration plays in the control of oncogenic mRNA interpretation and the advancement of cancer. To aid in cancer existence and growth, tumor cells deliberately increase the translation of particular oncogenic transcripts, but the mechanisms underlying are incompletely known. Here, we discover that intrahepatic cholangiocarcinoma (ICC) is substantially more likely to have N7-methylguanosine (m7G) tRNA alteration and its methyltransferase group members, METTL1 and WDR4 [45].

3.3. Oncogene Sloppiness in mRNA Translation

A highly conserved and well-regulated method for muscle growth is mRNA translation. Despite quality assurance systems, ribosomal frameshifting causes abnormal proteins to be produced in melanoma due to amino acid deficiency. This phenomenon's scope and underlying mechanisms are still unknown. The ribosomal frameshifting caused by tryptophan deficiency occurs often in cancer. The link between this event's sloppiness and the excessive activation of the MAPK pathway is strong. In primary cells, RAS activation promotes sloppiness, which is decreased in sloppy cells by pharmacological cease of the oncogenic Signaling pathways and is reversed in cells with attained resistance to MAPK pathway inhibition. Curiously, sloppiness results in abnormal peptide appearance at the cell surface, letting T lymphocytes to identify and specifically kill drug-resistant cancer cells. As a result, while oncogenes promote the growth and aggressiveness of cancer, they also reveal a weakness by inducing the synthesis of abnormal peptides [46].

4. Oncogenic AKTivation of Translation as a Therapeutic Target

Proliferation, cell growth, viability, and angiogenesis are just a few of the numerous biological processes that the AKT signaling pathway affects and which

are frequently changed in cancer. AKT regulates the different mechanisms of mRNA translation at each phase, from ribosome formation to translation start and extension, in order to control protein synthesis. Recent studies have shown that oncogenic AKT can alter how genes are regulated during translation, leading to cellular transformation. Oncogenic AKT signaling causes both broad changes in protein synthesis and goals achieved in the translation of particular mRNAs, and emerging technologies are substantially improving the ability to recognize and functionally organize these translationally dominated mRNAs into gene networks based on their regulatory mechanisms [47].

One example is the study by Li *et al.* (2021), which used ribosome profiling to identify translationally regulated mRNAs in ovarian cancer cells with activated AKT signaling. The researchers found that oncogenic AKT promotes the translation of mRNAs encoding proteins involved in cell cycle regulation, metabolism, and stress response pathways, while repressing the translation of mRNAs encoding proteins involved in cell adhesion and extracellular matrix remodeling. This study illustrated how emerging technologies such as ribosome profiling can identify translationally regulated mRNAs and provide insights into the gene networks that are altered in cancer cells with activated signaling pathways [48].

To control these translational networks, oncogenic AKT stimulates ribosome biogenesis, translation initiation, and translational extension, which is still under investigation. Nowadays, the bulk of therapies aimed at translational control concentrates on inhibiting eIF4E hyperactivity in order to prevent translation start. In cancers driven by oncogenic AKT, it will be crucial to ascertain whether combination blockage of ribosome biogenesis, translation process initiation, and translation elongation might provide increased therapeutic effectiveness [49].

5. Antisense Oligonucleotides (ASOs)

In 1978, antisense technology first appeared. Zamecnik and Stephenson prevented translation by using recombinant nucleic acid molecules [41]. Ever since, numerous advancements have occurred to improve the technology for medicinal uses, including changes to the core, sugar compounds, and backbone. Currently, Formivirsen (Vitravene), Mipomersen, and Macugen are the three oligonucleotide medications that have received FDA approval for use in clinical settings. IE2 gene is the targeted of the drug formivirsen, which is used to treat CMV retinitis in Cancer sufferers [50]. Antisense oligonucleotides can be single or double-stranded, 15 to 25 nucleotides long, and can base pair with RNA. Antisense oligonucleotides must first be capable of crossing the cell membrane to capable of bind to pre-mRNA, mRNA, and noncoding RNA in the nucleus or capable of bind to miRNA and mRNA within the cytoplasm. The MOA of the antisense oligonucleotides depends on the target region of the mRNA, pre-mRNA, noncoding RNA, and miRNA. Sometimes this target region is not accessible to the antisense oligonucleotide lowering its efficacy. As a result, terminal sequences are best to target with antisense oligonucleotides due to increased accessibility. Double-stranded

antisense oligonucleotide utilizes the RISC complex to degrade mRNA, pre-mRNA, noncoding RNA, and miRNA [51].

5.1. ASO Mechanisms of Action

Based on the area of the RNA sequence that is treated and the ASO chemical characteristics, ASOs can function through quite a variety of methods. ASOs select the mRNA segments target depending on how accessible they are to binding. It has been discovered that the best sequences are bulges of 10 bases period of at least, hairpins, internal cycle repetitions, terminal sequences, and sequential within those loops. The two most popular ASOs are single-stranded ASOs, which use a range of techniques to mute expression levels, and double-stranded ASOs, which use the RISC system to digest RNA. Mechanism of action of ASO following as

1) Inhibiting 5' cap development;
2) steric obstructive of protein translation;
3) Inhibiting the RNA splicing;
4) Stimulation of RNase H.

5.1.1. Inhibiting 5' Cap Development

By directing the ASO to segments in the 5' UTR, the development of the 5' cap can be avoided. As was shown when oligonucleotides made to attach the 5' cap inhibited the attachment of the translation initiation factor eIF-4, oligonucleotide adherence at the cap area of pre-mRNA hinders the interaction of proteins required for cap synthesis. All mRNA variants have a 7-methyl guanosine residue at the 5' end. EIF-4 interacts to this residue and joins forces with eIF4-G, which also is bound by the ribosome. Activation of the translation process is triggered by subunit eIF4-G interaction with eIF4-. As a result, eIF-4 binding inhibition prevents 5' cap dependent synthesis [52].

5.1.2. Steric Obstructive of Translation

Steric blockage of mRNA translation is often accomplished by creating ASOs that engage at or close to the mRNA sequence's start codon and prevent the translation equipment, such as the subunit of the ribosome, from interacting. These ASOs can impede RNA building, block access to pre-mRNA, and mRNA, but they do not cause the target mRNA to be degraded. Since these ASOs are not structurally bound by the requirement to integrate, they frequently have more extensive chemical alterations than those that must be included into RNAi machinery. These oligonucleotides can also function in a manner that is identical to that of the splice-switching oligonucleotides [52].

5.1.3. Alteration of Splicing

The precursor form of mRNA (pre-mRNA, which is created by the transcription of DNA, must be cut preclude non-coding introns that include or omit particular exons in order to create mature mRNA. Small nuclear RNAs and proteins in the spliceosome control this process. Additionally important to this process are

repeated regions in the splice joints and activator and silencer regions in the introns and exons. Disease can result from changes in the proper splicing pattern. DMD, or Duchenne muscular dystrophy, is a prime illustration of this. The protein dystrophin, which is necessary for the integrity of the sarcolemma membrane, has its translational reading frame altered by inappropriate deletions 5. Males with DMD become less mobile between the ages of 10 and 12 and pass away in their mid-20s from respiratory and/or cardiac failure 5. ASOs have the different capacity to restore the RNA by encouraging splicing that results in the accurate RNA sequence, which is insertion of the correct exons, and so prevent the splicing process from erroneously removing specific sequences in the DMD/dystrophin pre-mRNA. The sickness may then be lessened by producing the right form of the polypeptide from the typical mRNA sequence [53].

5.1.4. Stimulation of RNase H

The correct splicing pattern may alter, leading to the disease. Duchenne muscular dystrophy is a prominent example of it. Unsuitable removals affect the translational gene transcription of the protein dystrophin, which is essential for maintaining the sarcolemma membrane. Males with DMD lose mobility between the ages of 10 and 15 and die of respiratory and/or cardiac failure in their mid-20s. In order to prevent the splicing mechanism from incorrectly removing some sequences in the DMD/dystrophin pre-mRNA, ASOs have the exceptional capability to restore the RNA by promoting splicing that results in the proper RNA sequence, which is insertion of the right exons. By creating the proper protein shape, the illness may then be alleviated [54].

5.2. ASO Modifications

The following challenges have hampered the development of much more RNA-based therapies oligonucleotide upheaval vulnerability to nuclease destruction, off-target impacts, ineffective transport to the intended tissues or limited cellular uptake, and low affinity for the specific mRNA. To get over these restrictions and boost stability, affinity, specificity, and delivery while lowering the possibility of off-target effects, ASOs have undergone substantial chemical modification. Since unaltered ASOs possess a negative net charge, they cannot enter the plasma membrane and are instead destroyed by endonucleases in the bloodstream without these changes [55].

6. Ribozymes

RNA proteins having catalytic activity are called ribozymes. They can slice target RNAs and thus hinder gene transcription & translation and permit manipulation of genes [56]. Ribozymes have two domains to perform their activity: one domain through base complementarity binds to a section of the target RNA, and the other domain performs catalytic activity [57]. Hammerhead ribozymes are well defined and studied. These ribozymes have a preserved area of 15 nucleotides and are protected by three stems helical structure, and their activity facili-

tated by an contact between special sequences on the first and second stem [58].

In 1982 Kelly Kruger and his associates established the term ribozyme. Researchers first isolated ribozymes from *tetrahymena thermophilia*. Ribozymes have the catalytic ability of self-cleavage and RNA cleavage. Researchers have discovered the most notable ribozyme, the hammerhead ribosome, from the tobacco ringspot virus. The hammerhead ribozyme is shaped like a hammer with a plus strand and has a minus strand shaped like a hairpin. The helices of the ribozymes separate the catalytic portion, and the internal hairpin loop is on the reverse side of helix two. The minimal required structures for catalytic activities are helix two, loop two, and catalytic site. The minimal required design is a minizyme, and minizymes can infiltrate RNA within the nucleus better than full-length ribozymes. Hammerhead ribozymes with three helices bind the target mRNA by base pairing and cleave the target mRNA's phosphodiester bonds by transesterification. The hammerhead ribozyme cleaves the target mRNA is at a site rich in GUA, GUC, or GUU.

Therapeutic Applications of Ribozymes in Cancer

Larger ribosomal subunit of RNA is working as a peptidyltransferase as well (10 - 13). The significant working of spliceosomal smaller nuclear (sn)RNAs as a ribozyme in complex along with pre-mRNA to catalyze pre-mRNA splicing has also been planned. A ribozyme is an RNA molecule that can function as an enzyme even in the absence of any proteins. They have the remarkable specificity necessary to catalyse the breaking and/or formation of covalent bonds, which speeds up these events. The self-splicing Tetrahymena group I intron and the RNA moiety of RNAse P were the first examples of RNA acting as a catalyst (1 - 3). Since the identification of these two RNA enzymes, self-splicing group II introns of some microbes and plant mitochondria, hepatitis delta virus, and a satellite RNA from Neurospora mitochondria have all been linked to Transcription catalytic activity [59].

7. siRNA Treatment for Leukemia

SiRNA is an effective adjuvant treatment for Acute myeloid leukemia because it down-regulates the translation of Mcl-1 mRNA, decreases Mcl-1 mRNA's concentration, reduces the survival rate of HL-60 cells, and triggers apoptosis. Karami *et al.*, using qRT-PCR and western blotting, demonstrated that siRNA reduced translation of Mcl-1 mRNA and reduced Mcl-1 mRNA levels. 72 hours after post-transfection with siRNA, associated to the control, Mcl-1 protein levels decrease to 20.76 percent, and Mcl-1 mRNA levels reduced to 16.22 percent as compared to the control [60].

Karami *et al.* [61] evaluated if the downregulation of Mcl-1 protein and mRNA is toxic to cells by exposing cells with Mcl-1 siRNA for 24 and 48 hours and then examined the cells via MTT assay. The HL-60 cells showed an 81.21 percent survival rate at twenty-four hours and a 65.15 percent survival rate at 48

hours. HL-60 Cells had shown evidence of cytotoxicity within 24 hours and marked cytotoxicity within 48 hours relative to the control.

After Karami *et al.* [61] discovered that survival of HL-60 cells was dependent on the up-regulation of Mcl-1, they evaluated if the suppression of Mcl-1 mRNA and protein would hinder propagation. Karami *et al.* [61] treated experimental HL-60 cells with Mcl-1 siRNA more than five days and exposed the control HL-60 cells with NC siRNA for five days. Karami *et al.* [61] used trypan blue to evaluate the cell survival rate of the experimental and control HL-60 cells every twenty-four hours for five days. SiRNA significantly hampered the proliferation of experimental HL-60 cells compared to the control HL-60 cells. After 24-hours, the viability of the experimental HL-60 cell fell to 84.40 percent. Only 53.37 percent of viable experimental HL-60 cells were left by day five. Using ELISA death assay, they found that the cellular suppression of Mcl-1 was cytotoxic to the cell. They also extrapolated that the cell cytotoxicity was due to the suppression of survivin [61] [62].

The mitochondrial can respond to pro-apoptotic death signals and antiapoptotic signals. An example of pro-apoptotic death signals is bax, while anti-apoptotic signals are Mcl-1 and survivin. Bax is a pro-apoptotic signal because it facilitates the opening of mitochondrial outer membrane channels. Cytochrome c and Smac/DIABLO diffuse out of the open mitochondrial membrane channels into the cytoplasm. Once within the cytoplasm, cytochrome c makes a complex with apaf-1. The complex is apoptosome. The apoptosome attracts and cleaves procaspase nine into active caspase nine. Active caspase nine then activate executioner caspases 3, 6, and 7. Smac/DIABLO inhibits survivin from inhibiting caspase nine.

8. miRNAs Treatment for Leukemia

Through posttranscriptional regulation, miRNAs have been linked to the control of hematopoiesis and have been shown to play important roles in the pathogenesis and prognosis of leukaemia [63]. Leukemia may develop because of haematological system disruption brought on by miRNA dysregulation. Leukemia cytogenetic and molecular subtypes can be distinguished by miRNAs. These miRNAs can either be recognised miRNAs related with leukemogenesis that have been shown to have tumour suppressor or oncogenic activity, or they can be newly identified miRNAs with yet unknown functions thanks to high-throughput sequencing.

To predict patient prognosis and the clinical response to treatment, one can use the expression profile of cancer miRNAs. The majority of miRNAs are restricted to the cell, although circulating miRNAs have been found in various bodily fluids and serve as novel biomarkers for solid and hematologic malignancies.

8.1. miRNAs as Tumor Suppressors in Leukemia

MiRNAs are supposed to be critical to the progress and propagate of human ma-

lignancies including leukemia. The miRNAs that have a role in tumor are known as oncogenic miRNAs, and recent research suggests that miRNAs can act as tumor suppressors and oncogenes. Some miRNAs exhibit lower expression in malignant cells during oncogenesis. These miRNAs are referred to as tumor suppressor genes. Oncogenes or genes that regulate cell differentiation or death are frequently negatively restrained by tumor suppressor miRNAs, which prevent the growth of tumors. Several miRNAs are currently thought to act as tumor suppressor genes [64].

Some miRNAs may target important AML, ALL, CLL, and CML genes, acting as tumor suppressors in leukemias. For instance, miR-15 and miR-16 cause apoptosis by concentrating on the mRNA of the antiapoptotic B cell lymphoma 2 (BCL2) gene, a significant contributor to a amount of human malignancies, including leukemias and lymphomas. Since these two miRNAs downregulate BCL2 at the posttranscriptional level, Cimmino *et al.* [65] demonstrated that the expression of miR-15a and miR-16-1 is negatively connected to BCL2 expression in CLL. According to these results, miR-15a and miR-16-1 could be used to treat malignancies that overexpress BCL2. Calin *et al.* (2021) used miRNA sequencing to show that the transcription of miR-16 and miR-15 is decreased in human CLL, and that this decrease is also related to the clinical and biological characteristics of CLL [66].

8.2. miRNA as Diagnostic and Prognostic Biomarker

There are four types of leukemia. The four types of leukemia are Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), and Chronic lymphocytic leukemia (CLL). It can be challenging for clinicians to differentiate myeloid leukemia from lymphoid leukemia. MiRNAs can be used as biomarkers to determine myeloid leukemia from lymphoid leukemia. MiRNA can distinguish myeloid leukemia from lymphoid leukemia with 95 percent efficiency because myeloid leukemia's and lymphoid leukemia's miRNA profile is unique. Cancer cell in Acute myeloid leukemia typically shows downregulation of miRNA-223 and upregulation of miRNA-128a and miRNA-128b (Yeh *et al.*, 2016). The following are miRNA biomarkers that can differentiate Acute myeloid leukemia (AML) from Acute lymphocytic leukemia (ALL): miRNA-23a, miRNA-27a, miRNA-199b, miRNA-221, and miRNA-223.

PCR and flow cytometry can detect leukemic blasts. The problem with using PCR and flow cytometry is that leukemic blasts typically occur late during a relapse [67]. The time chain reaction and flow cytometry detect the leukemic blasts can be too late for preemptive therapy. The ability to diagnose minimal residual disease improves treatment options and outcomes for patients who will experience leukemic relapse. Biomedical researcher proposes using miRNA because it is not expensive, noninvasive, and can detect minimal residual disease with accuracy. Early research indicates that researchers can monitor miRNA profiles after chemotherapy and detect AML-associated miRNA serum profiles. The

problem with earlier research on using miRNA to detect AML-associated miRNA serum profiles is the lack of consistency.

9. miRNA Therapeutics

MicroRNAs (miRNAs) are small and evolutionarily preserved noncoding RNAs having 20 - 24 nucleotides in length. Transcription and maturation are two ways for generating miRNA. The mature miRNAs are integrated into the miRNA-induced silencing complex (miRISC), a complex of miRNAs and Argonaute (Ago) proteins, to control miRNA-induced silencing of target mes-senger RNAs (mRNAs) [68]. It is assessed that miRNAs regulate over 60 percent of human protein coding genes [69]. The benefit of using miRNA is that it can target multiple targets within a pathway or a common biological process. Resistance to miRNA is not likely due to its ability to target more than one target oncogenic mRNA at once. Researchers better understand how mutation results in acute myeloid leukemia and design miRNA that can target these oncogenic mRNA.

MiR-29b is a type of miRNA. A mice model implanted with human acute myeloid leukemia and in vitro model has shown that MiR-29b can be an effective treatment for leukemia and has an efficacy of delivery greater than 100-fold. MiR-29b indirectly or directly inhibited CDk6, SP1, FLT3, DNMTs, and KIT in mice models implanted with human acute myeloid leukemia and in vitro [67]. Other miRNAs effective in treating leukemia are miR-22 mimic, miR-21/miR-196b antagomiRs, miR-126 antagomiR, and miR-181a mimic.

Researchers are currently using Pevonedistat in clinical trials due to its ability to inhibit oncogenic miR-155 in FLT3-ITD+ in acute myeloid leukemia cell lines [67]. Pevonedistat mechanism of action reduces oncogenic miRNA levels by targeting miRNA pathways. Pevonedistate reduces leukemic phenotype expression in mice model and in-vitro studies.

There currently is no miRNA therapy in clinical trials due to ineffective delivery methods. Nuclease within the blood rapidly degrades synthetic miRNA, and delivery of miRNA to bone marrow is difficult. A large quantity of miRNA is required to deliver miRNA to the bone marrow. Administering such a large quantity of miRNA runs the risk of the immune system identifying the miRNA as foreign. The immune system recognizing the miRNA as foreign can trigger interferon and antibodies that sequester and inactivate the synthetic miRNA. Current delivery methods include "liposomes, nanoparticles, and LNAs" [67].

10. Antisense Oligonucleotides Therapeutics

18 - 30 nucleotides long, chemically arranged, single stranded nucleic acids are called antisense oligonucleotides (ASOs) [70] [71]. ASOs act as small drug molecules that bind different RNAs and modulate gene expression by involving with different steps such as complementary base pairing, splicing, transcription, or translation through various mechanisms [72] [73] [74]. Based on their action, ASOs are categorized into two main types, the first promotes RNase H1 cleavage

and Argonaute 2 degeneration and the second by steric hindrance-mediat modulation, known as steric block [71] [75]. RNase H1-based ASOs act on nuclear transcripts such as pre-mRNAs and lncRNAs because they are not degraded by siRNA. Steric block ASOs are used to regulate the various steps of RNA processing and interaction of the target RNA [72] [73]. Studies have shown that applying ASOs to modulate and target microRNAs, noncoding RNA, as well as splicing of transcripts, are systematic ways to modulate protein synthesis [76].

26-mer bcr-abl antisense oligonucleotide has shown to be an effective treatment option for chronic myelogenous leukemia. Chronic myelogenous leukemia results from a hybrid tumor oncogene—the hybrid tumor oncogene results from a bcr-abl translocation event between chromosomes 9 and 22. The Abl is usually under tight control when it is on chromosome 9. However, once on chromosome 22, it is no longer under control and proliferates uncontrollably. Skorki has shown that 26-mer bcr-abl antisense oligonucleotide can retard chronic myelogenous leukemia in mice [62]. Mice treated with 26-mer bcr-abl antisense oligonucleotide had decreased CD10+ cells. Experimental mice treated with 26-mer bcr-abl antisense oligonucleotide survival time was twice that of control mice that researchers did not treat with 26-mer bcr-abl antisense oligonucleotide, and experimental mice only had RT-PCR evidence of having chronic myelogenous leukemia [62].

11. Ribozyme Therapeutics

Ribozyme can cleave target RNAs and thus hinder gene transcription & translation and allow manipulation of genes [56]. Ribozymes have the ability to bind specifically recognized RNA sequences and then cleave them. These specific properties make this class of molecules to be used in cancer therapy as RNA drugs. Once the RNA sequence is cleaved, ribozymes de-attach from the sequence and can bind and cleave other RNA sequences [77].

Ribozymes are an effective treatment for leukemia as they suppress p210 by a factor of two compared to antisense oligonucleotides. P210 is one form of an oncogenic bcr-abl mRNA, and bcr-abl mRNA is generally present in all patients with chronic myeloid leukemia (CML). K562 cells exposed to anti-B3A2 ribozymes showed partial inhibition of p210. However, Synder *et al.* [78] completely inhibited p210 with DNA-RNA hybrid ribozyme. RNase H's reduced capacity to degrade the hybrid ribozyme improved its efficacy.

12. Combination Therapy

Currently, RNA-based therapeutics have been remodeled by a new approach called combination therapy by using ASOs to modulate protein synthesis in various diseases such as cancer [70] [73]. Skorski knew that MYC presence is mandatory for the bcr-abl transformation of hematopoietic cells. Therefore, he treated mice with SCID and CML with 26-mer bcr-abl antisense oligonucleotide and a 26-mer c-myc antisense oligonucleotide and other mice with just 26-mer

bcr-abl antisense oligonucleotide. As 26-mer c-myc antisense oligonucleotide would inhibit MYC mRNA and 26-mer bcr-abl antisense oligonucleotide would inhibit bcr-abl mRNA. His experiment showed that mice treated with 26-mer bcr-abl antisense oligonucleotide and 26-mer c-myc antisense oligonucleotide had a longer survival time than mice treated with only 26-mer bcr-abl antisense oligonucleotide. Skorski's experiment supported a multidrug approach when using 26-antisense oligonucleotides.

13. Drawbacks

The kidney rapidly clears antisense oligonucleotides, ribozymes, siRNA, miRNA, and nuclease degrades them. Only when administered within the central nervous system does the kidney not filter them out. The kidney does not easily filter antisense oligonucleotides, ribozymes, siRNA, and miRNA from the central nervous system, as it is a closed system. The use of RNA-based therapeutics and their clinical applications are restricted because of their absence of stability, toxic effects, and other physiological processes that limit their intracellular delivery [79] [80] [81]. The other drawback is that the nuclease will also degrade antisense oligonucleotides, ribozymes, siRNA, and miRNA. Using a short-term viral vector that administers antisense oligonucleotides, ribozymes, and siRNA is not advantageous as short-term viral vectors would only distribute them within the cytoplasm. Researchers should focus on using long-term viral vectors that would insert the genetic information of antisense oligonucleotides, ribozymes, siRNA, or miRNA in the organism genome. Suppose researchers inserted the antisense oligonucleotides, ribozymes, siRNA, or miRNA genetic information into the genome. Researchers would not have to worry about the kidney clearing them from the bloodstream and enzymes degrading them. There will be steady production of antisense oligonucleotides, ribozymes, siRNA, or miRNA even though they are removed from the blood and degraded by enzymes.

14. Conclusion

This review clarifies the use of gene interference in cancer treatment. As a tool to target genes from the perspective of a person's genetic history and lifestyle, RNA interference holds enormous potential in the area of precision medicine. A summary of current antisense and RNA-based medications that are being used in clinical studies is provided. Since the first publication of antisense suppression of genes, new chemistries have been devised to increase the transport, specificity, affinity, and nuclease tolerance of the oligonucleotides while lowering their toxicity. One evaluation cannot cover the entire timeline of all the work performed for each of these. However, the goal of this analysis is to provide a general sense of the difficulties and advancements facing the subject. Many different diseases, particularly malignancies, are being treated with antisense oligonucleotides. ASOs are faced with difficulties while treating cancer since non-specific targeting might increase clotting times and cause unfavorable immune activation. The

delivery of ASO is inefficient and poor, which allows some cancer cells to evade treatment and hinders its usage in cancer treatment. ASOs still have a way to go before they can be used as effective cancer treatments, despite the fact that several chemical modifications, such as nanoparticle transports, have been created to get around these restrictions.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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