RNA Drugs and RNA Targets for Small Molecules: Prospects and Challenges

By

Md. Raihanul Islam
15346033

A project submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
Brac University
August 2021

Declaration

It is hereby declared that

1. The project submitted is my own original work while completing the degree at Brac University.
2. The project does not contain material previously published or written by a third party.
3. The project does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all the main sources of help.

_____

Student Full Name: Md. Raihanul Islam
Student ID: 15346033

Approval

The project titled "RNA Drugs and RNA Targets for Small Molecules: Prospects and Challenges" is submitted by Md. Raihanul Islam (15346033) of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 28 August 2021.

Supervised By:

Easin Uddin Syed
Lecturer (On Study Leave)
School of Pharmacy
Brac University

Approved By:

Program Director:

Dr. Hasina Yasmin
Program Director and Assistant Dean
School of Pharmacy
Brac University

Dean:

Dr. Eva Rahman Kabir
Dean
School of Pharmacy
Brac University

Ethics Statement

This study does not involve any kind of human or animal trial as well as any types of harm.

Abstract

RNAs are the small molecules that have been inferred in many human diseases and they are important for gene regulation, gene expression, and cellular information transfer. The highly structured elements of the messenger RNA and non-coding RNAs are essential for the functions of the drugs. The small molecules combined with these RNAs provide a possibility to modulate diverse cellular processes therapeutically which incorporate them, who are linked to undruggable protein targets. However, only the linezolid antibiotics are clinically used among all other human-designed small molecules which target the RNA. With the identification of a huge number of small-molecule RNA ligands, the interest in this discipline is expanding. The purpose of this review is to explore the recent advancement with RNA drugs & target RNAs and the challenges for discovering suitable target structures and selective small-molecule binders.

Keywords: RNA drugs; RNA small molecules; gene slicing and editing; RNase; RNA molecule and Protein; RNA therapeutics.

Dedication

Dedicated to my parents, all the faculty members and friends who helped me throughout the undergraduate phase.

Acknowledgments

Firstly, I am grateful to my Almighty Allah for the decent health and wellbeing during the project which was vital to complete the project work on time.

It is a great pleasure to express my deep sense of thanks and gratitude to my supervisor, **Easin Uddin Syed** (Lecturer (On Study Leave) School of Pharmacy, Brac University) for giving me this golden opportunity to do this wonderful project. His prompt inspiration at every stage of my project work and timely suggestion with kindness, dedication, scientific approach, constant guidance, collaboration, and enthusiasm make me enable to finalize the project.

My special thanks to honorable Professor **Dr. Eva Rahman Kabir** (Dean, School of Pharmacy, Brac University) whose inspiration helped me throughout the project.

Also, I take this opportunity to express my gratitude to all the faculty members and lab officers for their help and support.

Last but not the least, I would like to express my greatest regards to my parents for their co-operation, continuous support, unconditional love throughout my life.

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List of Acronyms

ASO	Antisense Oligonucleotide
BERA	Bioengineered RNA Agents
FDA	Food and Drug Administration
asRNA	Antisense RNA
HCV	Hepatitis C Virus
HPLC	High Performance Liquid Chromatography
LNP	Liquid Nanoparticle
miRNA	micro RNA
ncRNA	noncoding RNA
PD	Pharmacodynamics
Pre-miRNA	Precursor miRNA
RISC	RNA Induced Silencing Complex
TCR	T cell Receptor
sRNA	small RNA
SARS	Severe Acute Respiratory Syndrome
rRNA	Ribosomal RNA
shRNA	short hairpin RNA

siRNA	small interference RNA
qPCR	quantitative Polymerase Chain Reaction
HD	Huntington Disease
CRISPR	Clustered Regulatory Interspaced Short Palindromic
TAR	Transactivation Response
mRNA	messenger RNA
PK	Pharmacokinetics
DC	Dendritic Cells

Chapter 1

Introduction

The RNA has developed as a novel class of therapies that may allow for the re-targeting of mutated targets, potentially expanding the spectrum of druggable targets for not only from proteins but also to the RNAs and the genome. To begin with, suitable RNA molecules, such as aptamers, can be used to block the current protein targets as well as previously unidentifiable proteins to induce the targeted pharmacological effects. Second, RNA entities including antisense RNAs (asRNAs), small interfering RNAs (siRNAs), microRNAs (miRNAs) and other forms of short RNAs (sRNAs) may target mRNAs and non - coding RNA's directly to silence the expression of a gene of interest or in managing diseases. Finally, by utilizing a suitable guide RNA (gRNA) and other required components, the sequence of a gene controlling illness beginning or development can be directly changed to accomplish a full elimination of the disease for patients (Khorkova & Wahlestedt, 2017; Zhou & Rossi, 2017).

The first-of-its-kind mRNA-targeting patrician (ONPATTRO) was approved for clinical use by the US Food and Drug Administration (FDA) in August 2018, highlighting the promise of RNA therapeutics. When compared to traditional small-molecule and protein therapies, RNA therapeutics have not only different modes of action, but also different pharmacokinetics characteristics and chemistry. As a result, developing new RNA therapies has proven to be extremely difficult, and only a few nucleic acid medicines have been authorized for therapeutic application in the last two decades. As long as RNA research and medication development has struggled to distribute RNA molecules, the proper RNA molecules have been neglected. In RNA research and medication development, chemically engineered or synthesized oligonucleotides or RNA "mimics" are presently dominant, and many have been approved for

clinical use by the Food and Drug Administration (FDA). However, these "mimics" are ornamented with chemical changes. (Crooke et al., 2018; Lundin et al., 2013). It distinguishes them from natural RNAs, which are transcribed from the genome and coiled in living cells with no or few post-transcriptional alterations. Protein research and treatment have been mostly effective when biologic or recombinant proteins are produced and folded in living cells rather than polypeptides or proteins synthesized in vitro through peptide chemistry .Recent efforts have focused on developing new biotechnologies for generating biological and bioengineered RNA agents (BERAs) inside of the cells. (Leader et al., 2008; Pereira et al., 2016).

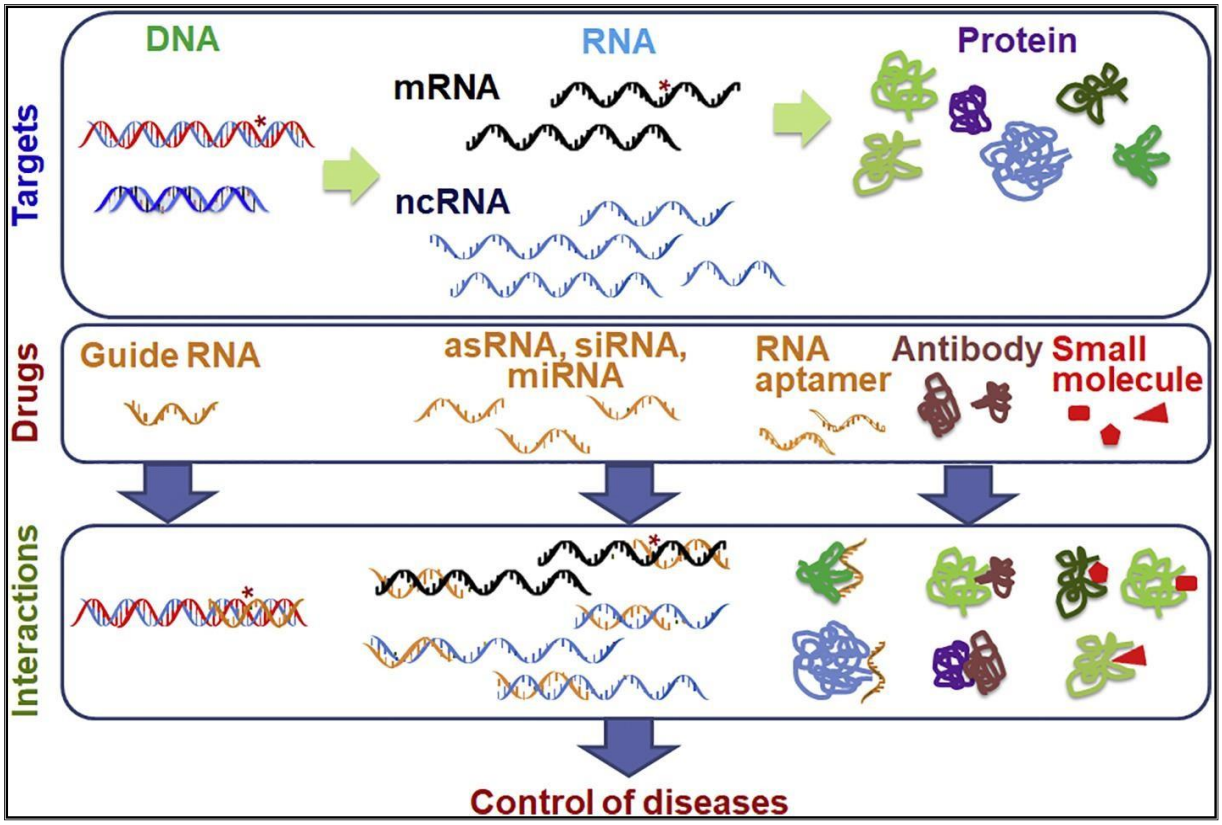

Figure 1 : Expanding the Range of Druggable Targets with RNA therapy (Watts & Corey, 2012).

For some years now, RNA has been considered to be a molecule full of twists and turns, but the true power of its capacity to control life has, unfortunately, been underestimated on many times. The amazing discovery that RNA was generated in length and then shortened to code

for protein was discovered by Sharp and Roberts. For many years, it was believed that RNA was a passive molecule that served as a protein's binding partner, but it has since been found that it may act as a catalyst, too. (Corey, 2007). Before this discovery, the only way to successfully block gene expression in *C. elegans* cells was to use RNA, which the Fire and Mello labs found could be injected into the cells. According to the conventional view, RNA was formerly thought to be just a transporter of genetic information, but the current findings of RNA interference (RNAi) indicated that RNA may also control gene expression. The finding of Fire and Mello was remarkable in three ways. To begin with, it revealed a previously unknown natural regulatory mechanism that has the potential to use Watson-Crick base-flexibility pairings to control gene expression. Next, it offered an essential fundamental research tool for researching gene function (inhibiting gene expression is frequently a good way to look at a gene's function). Finally, it offered the faint hope that this very simple method may be used to regulate disease gene expression in human cells and generate a new class of therapies (Mello, 2007).

The purpose of this review is to explore the recent advancement with RNA drugs & target RNAs. Furthermore, review will explore the challenges for discovering suitable target structures and selective small-molecule binders.

RNA Drugs

The potential drugs can be used therapeutically for gene silencing, editing by the RNA molecule, and with the help of a new biological process. The protein drugs and the small molecules usually target the RNA aptamers which are straightly blinded to the cell surface and intracellular proteins. In addition, to control the gene expression for the treatment of disease and gene slicing the ASOs, siRNAs, and miRNAs duplicates can be delivered to the cells for targeting the functional ncRNAs and mRNA through complementary base parings. For

vaccination or protein replacement therapy the sense RNA or mRNA molecule is put into the cells after that it is translated to target proteins (Sahin et al., 2014). The gRNAs and the other necessary components can be used to directly change the initiation and progression of the genetic sequencing dictating diseases to attain the elimination of the disease. The most unique character of RNA is that it can interact with the most important forms of biological macromolecules which are DNAs, RNAs, and Proteins. Moreover, RNA therapeutics is developing rapidly with the hope to enlarge the field of druggable targets which also consists of the undrugged transcripts and genes and the conventional proteins. The RNA drugs are anticipated to act on the intracellular targets primarily and the RNA molecules show the 'undrug' like PK characteristics when it is differentiating with the compounds with a small molecule.

Unmodified RNAs are likely to be digested by nonspecific RNases that are prevalent in blood as the polymeric molecule most of the time consisting of a sequence of many ribonucleotides that exist in four distinct nucleobase configurations (Tollervey & Houseley, 2009).

Because RNA is a big negatively charged molecule, it is very difficult for it to penetrate the cell membrane. Additionally, exogenous RNAs must enter cells to avoid destruction by large class sizes of intracellular endosomal entrapment and RNases, as well as get access to the targets for exerting the pharmacological effects. There are many RNA analog medicines that have been authorized by the FDA for the treatment of human illnesses, and many more are in the clinical trial phase due to a better knowledge of the RNA molecules' action processes and newly created drug capabilities. In addition, to inhibit the protein targets pegaptanib, the first RNA aptamer drug reinforces the concept of using drug. ASOs are now known as most successful RNA drugs by following specific chemical modifications to upgrade the level of silencing efficacy, binding affinity , targeting and metabolic stability (Crooke et al., 2018).

Besides, the recently approved two siRNA drugs are givosiran and patisiran which supports the potential RNA therapeutics and testifies the benefit to develop new approaches for improving the pharmacodynamics (PD) and pharmacokinetics PK properties of RNA drugs. The drug efficacy and safety and the delivery issues has made the discovery of modern drugs suffered. Again, to develop the small molecules-based drugs and bio-therapeutics the therapeutic applications of the nucleic has become a topic of great interest and for these studies are going on with plasmid DNA and oligonucleotide drugs. In contrast, there are high opportunities for employing RNA-based approaches due to the improvement in recent technologies. To treat the disease or antigens for the development of vaccines the therapeutic genes are expressed by the mRNA with the aim to provide immediate translation in target cells. The delivered transcripts cause to compromise the efficacy of treatment and restricted gene transient expression for the rapid degradation due to the presence of RNAase. To improve the stability of RNA major efforts are taken and special monitoring is done for delivery issues which includes nanoparticle, polymers and lipids for encapsulation of RNA molecules and also targeting the dendritic cells (DCs) that is also called antigen-presenting cells (APC) (Lundstrom, 2018). The delivery of RNA is applied by the RNA virus which can be self-amplifying RNA molecules. The threat posed by the pandemic pathogens is demonstrated the COVID-19 pandemic. The pandemic means the emerging pathogens that are able to spread quickly in lack of protective immunity. The factors like ecological and sociological has helped the urgency of zoonotic outbreaks whereas human oriented pandemics has increased by the biotechnological factor. The advancement in the editing technology and gene synthesis the actors with malicious wants to eradicate the pathogens for deliberate release. To counter the infectious disease threats in the pandemics the vaccines works as a powerful tool but it takes

more than 10 years to develop the vaccine from the preclinical phase to licencing (Sandbrink & Shattock, 2020).

Classification & Characteristics of RNA Therapy

RNA-driven therapies are divided into two categories: analogs or RNA molecules that can be directly utilized as potential therapeutic medication (Kole et al., 2012) and RNA-driven medicines (Donlic & Hargrove, 2018). Firstly, several RNA medications, ASO or antisense RNAs are authorized for treatment of several human diseases by the Food and Drug Authority (FDA). For example (GRA) (Grandwicz and other drugs). A broad array of known and therapeutic potential targets, including genes, transcripts, and proteins, were not accessible to small molecules and proteins were very selective in their action. However, RNAs are prone to serum RNAase catabolism and have to overcome the cell membrane barriers in an intracellular way. RNAs are not accessible orally, like protein therapies, thus RNAs and protein medicines are generally given by alternative methods, such as intravenous or subcutaneous injection. The RNAs and the RNAs are not available orally. That differs entirely from several tiny, inorganic AMC medications such as lithium carbonate and other different organic compounds that are beneficial or agreeable to patients and are mostly orally administered. Secondly, conventional compounds with large structural diversity and drug-like physiochemical and PK characteristics are favored to bind to highly organized RNA targets and alter them (Lee et al., 2017). After the RNA goal has been identified it is possible to pick correct medicine similar small molecules for additional preclinical and clinical studies to determine effectiveness and safety profiles and to determine the RNA–small molecules interactions. This concept is supported by numerous antibiotic drugs, including natural and semi-synthetic aminoglycosides (Demeshkina et al., 2012), tetracyclines as well as clinically approved synthetic oxazolidinones to mechanistically bind RNAs to interfere with protein-synthesizer in the 30S or 50S subunits for the control of

infections. There are thus considerable efforts being made to find suitable targets for RNAs and evaluate novel short molecules like RNA for the betterment of different many human illnesses.

The structure as well as chemistry matters regarding the performance of RNAs. Whether a single or double-stranded RNA targeting oligonucleotide affects significantly its performance. ASOs are single, whereas siRNAs are double-stranded and have a meaning and an anti-strain. In a siRNA, the antisense beam is the phenomenally active movement and the sense beam can be regarded as a "drug delivery system' that carries the antisense beam to the endonuclease intracellular RNA AGO (Crooke et al., 2018). After systematic management, ASOs containing phosphorothioate (PS) are widely distributed. Amphipathic and binding proteins in the serum, cell surface, and intracellular are single-strain PS-replaced ASO. These interactions promote the absorption and dispersion of cells, allowing these types of ASOs to be distributed to most of the body's tissue and through almost all routes of saline administration. In contrast, dual-strand siRNAs, which also include polyanions and hydrophilic, do not attach to serum proteins and are quickly excreted; thus, they must either be formulated into Lipids or other nanoparticles or chemically altered and combined to a mode that interacts in an effective cell tissue delivery with a high-capacity surface receptor (Dowdy, 2017).

Meanwhile, different RNAs targeting oligonucleotides in a particular chemical class only differ in sequence, the physicochemical features of the individual groups are the same, therefore having shared pharmacokinetic and biological characteristics. However, each chemical class differs from that of scientifically unsuited modifiers, which may result in significant power changes, pharmacokinetics, and general chemical class impacts compared to 20 MOEs and even minor variations in scientists that are not chemically comparable in the field. Thus, the

chemistry of RNA-target oligonucleotides must be defined accurately in the finding of several human illnesses. (Bennett, 2019).

RNA Targets

RNA being a small molecule could be very advantageous for targeting drugs. The three differential structure is used for the receptors and enzymes that follows the change of occupancy which is driven from the functional sites. The pathological mechanisms which are related to disease states of the RNA can be analyzed from tissue or liquid biopsies generally (O'Rourke & Swanson, 2009). Through this study, it can understand that the molecular recognition of RNA by small molecules and the design of the bioactive small molecules along with the materials used for the research of RNA like target validation, management, and selectivity (Sztuba-Solinska et al., 2019).

RNA has played a vital role as a tiny molecule of individual drug target from the very beginning of modern medicine. Streptomycin had become the first-line treatment when it was found to be selected by the bacterial ribosome for *Mycobacterium tuberculosis* which used to be a devastating disease causing the patients to succumb to the infection. In Neosporin the active ingredient is the neomycin B, an aminoglycoside antibiotic that is the natural product of the *Streptomyces* species of bacteria. There is an abundance of use of the aminoglycosides based antibiotics which are mainly the derivatives of these as well as they have limited use until they are modified but can be used in broad-spectrum effectively (Disney et al., 2008) However, there are a quite number of ligands which targets the RNA and have proceeded to the clinical phenotypic screens to find the active antibacterial. To have a better idea about the mechanism of the action of the engaged targets is to be identified by the phenotypic screens and this creates a huge problem with the study. The assays' target validation tools are intended to investigate protein-centric pathways. To example, in order to identify a target for a small chemical, the

pooled CRISPR and shRNA hairpin libraries must knock down the ORFs; yet, it may also drive the ORF's expression. (Datlinger et al., 2017).

The tissue complexity and organism increase the complexity of the gene expression regulation through the RNA metabolism. For the pharmacological intervention the regulation of the gene expression provided by the brain cell through alternative RNA processing and small noncoding RNAs. The malfunction of the RNA processing event causes multiple human diseases for instance, for the aberrant mRNA processing, 15% of the human genetic disorder is caused. RNA has been recognized as a well-established therapeutic target since the ribosomal subunit has been designated as the antibiotic receptor, and an antisense oligonucleotide is utilized to inhibit gene expression. The particular mRNA sequence is intended to generate Watson-Crick base pairs when combined with complementary oligonucleotides. This method is used to inhibit the production of a certain gene product. The technology's principles have been validated by Vitravene, the first antisense medicine to get FDA clearance, and new drugs are continuously being developed. RNA interference is an intriguing new technique that utilizes short double-stranded RNA duplexes to achieve a higher degree of targeted gene silencing. (Filipowicz, 2000).

Given the role of improperly folded RNA structures in illness, it is critical to develop drugs that restore their functioning or enable their study in healthy and sick people. Beyond simple molecular recognition, it is important to target the RNA. Drugs targeting RNA must be able to reach the target RNA's structural components, even if they are in inhibitory structural relationships with other biomolecules. For example, competition with RNA-binding proteins (RBPs) or the RNA itself may make access difficult during blood brain penetrance. Additionally, selected agents must be capable of disrupting on-target RNA while avoiding excessive binding to off-target transcripts. Binding to a particular transcript is also affected by

the degree to which competing RNA motifs are expressed, which is linked with tissue- or compartment-specific expression. Additionally, compounds must be capable of inducing observable biological changes, which requires interaction with RNA at a functional location. Sequence complementarity through antisense oligonucleotide (ASO) hybridization with a target RNA strand is a standardized method for influencing RNA function. The resulting antiparallel base-paired duplex may either disrupt the normal folding of the target RNA, leading it to interact with other biomolecules, or attract endogenous cellular machinery to degrade the RNA (Bennett, 2019). These interactions, however, are constrained by the thermodynamic and kinetic energy barriers associated with folding/unfolding or hybridization of both the target RNA and ASO's natural conformations, which limits their efficacy against highly structured RNAs (Bernat & Disney, 2015). As a result, poorly structured RNAs are the greatest candidates for the antisense-based method. Apart from the difficulties inherent in establishing meaningful molecular interactions, oligonucleotides have been shown to have poor in vivo properties, resulting in limited delivery strategies, biodistribution, and tissue penetrance, as well as to cause a variety of adverse effects, including immune activation, thrombocytopenia, and hysteria (Chi et al., 2017).

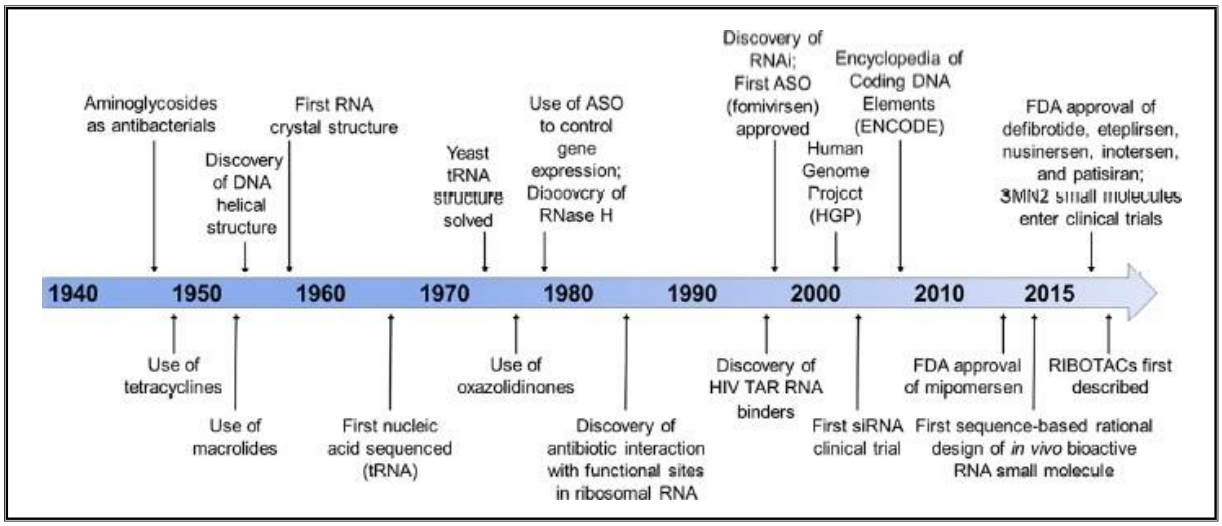

Figure 2: Timeline of major developments of RNA-targeting. (Costales et al., 2020)

Chapter 2

RNA interférences & Relevant RNAi Agents

RNA interférence

RNA interference is an increasingly preserved technique in which ncRNAs influence target gene expression at the post-transcriptional stage in eukaryotes. MiRNAs achieved from the genome and siRNAs formed from externally injected double-stranded RNAs are the examples of RNAi molecules (dsRNAs) (Ambros, 2004). The miRNA coding genes are first generated as primary miRNA transcripts, or pri-miRNAs, by RNA polymerase II in the nucleus. It is further refined into smaller precursor miRNAs (pre-miRNAs) by the ribonuclease (RNase) III called Drosha. Some pre-miRNAs, in contrast, are removed straight from the protein-coding gene's introns. After being transported from the nucleus into the cytoplasm through Exportin-5, pre-miRNA molecules are converted into double-stranded miRNA molecules by the cytoplasmic endoribonuclease Dicer. After being unwrapped from miRNA duplexes or siRNAs derived from dsRNAs, the single-stranded guide miRNAs or siRNAs are packed into

miRNA- or siRNA-induced silencing complexes (RISC or miRISC), which then selectively respond to target mRNAs via flawless or flawed base-pairing interactions resulting from targeting RNA degradation or translation repression. (Yu et al., 2020). RNAi is engaged in practically all cellular processes, including viral infection defense, cell transformation, and disease progression, by suppressing or adjusting target gene expression (Ambros, 2004; Bartel, 2009). Some miRNAs or the entire miRNome profiles appear to be significantly dysregulated in diseased cells compared to normal cells, suggesting that they could be a potential biomarker for diagnostic or prognosis. Additionally, functional miRNAs essential for illness progression could be used to design new therapeutic approaches (Bader et al., 2010). On the one hand, disease-causing miRNAs are overexpressed in diseased cells and promote disease initiation and progression may be blocked or silenced to accomplish disease control. MiRNAs that have been depleted in diseased cells or are capable of suppressing disease initiation and progression could be reintroduced to the cells to maintain disease progression (Yu et al., 2019).

Relevant RNAi Agents

Researchers have used different RNA molecules to control gene expression and modulate the RNAi process. They've discovered regulatory miRNAs derived from the genome and the importance of those miRNAs in human diseases. This knowledge has facilitated the development of many RNA molecules that control the RNAi process or imitate miRNA action in order to regulate target gene expression, investigate gene function, and regulate cellular processes and disease (Bader et al., 2010). Examples of RNAi modulators that contain various forms and degrees of chemical changes include synthetic asRNAs, dsRNAs, siRNAs, and miRNA mimics (Bramsen & Kjems, 2012). Moreover, “natural” RNAi molecules, also known as BERAs, have been transported into human cells for the regulation of target gene expression, and these molecules are synthesized and folded in bacteria using recently discovered RNA

bioengineering techniques (Duan & Yu, 2016). Non-viral or viral-based miRNA or short-hairpin RNA (shRNA) expression techniques are often used in vitro and in vivo RNAi research and medication development (Brake et al., 2008).

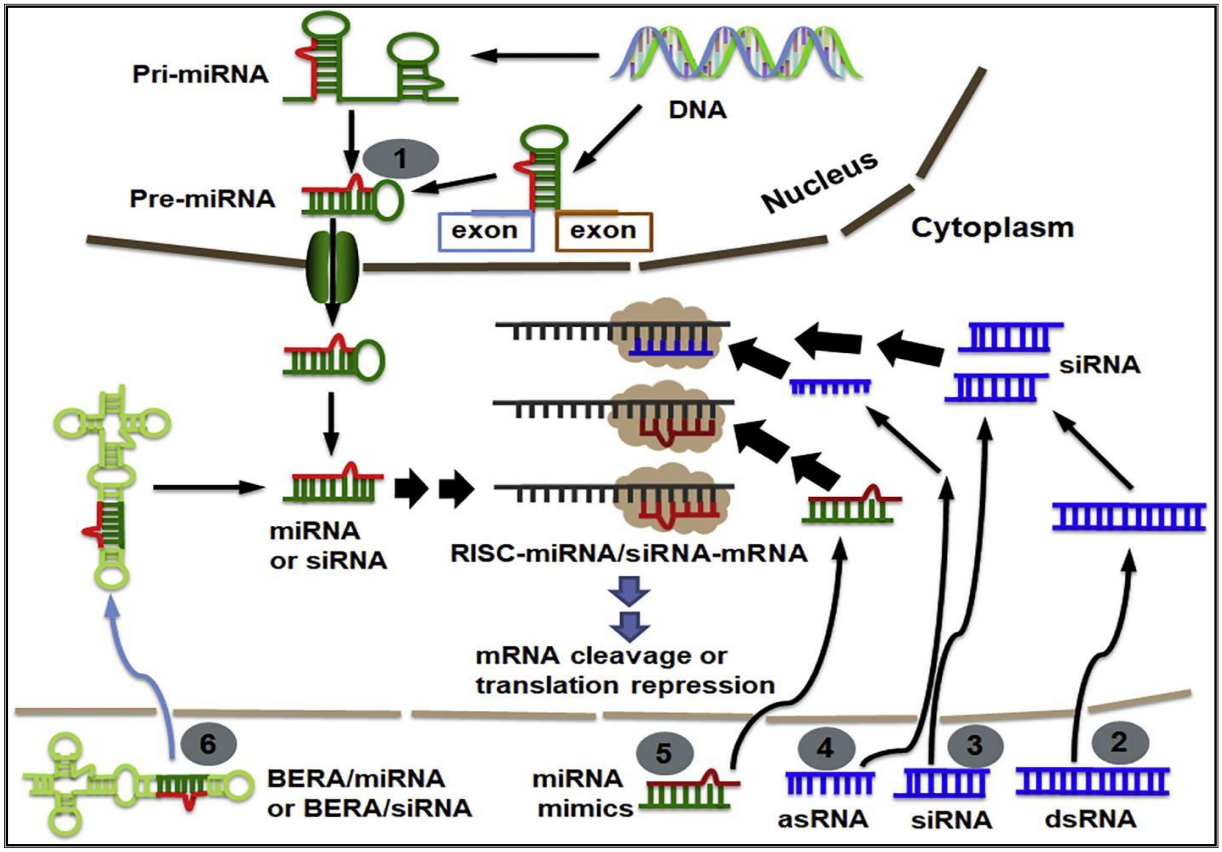

Figure 3: Cellular RNA interference pathway & manipulation with various types of RNA Agents.(Yu et al., 2019)

Advantages of RNA Interferences

Prior to the invention of RNAi in 1998, antisense technology based on nucleic acids had been used for many years to inhibit gene expression on a sequence-specific basis. The primary benefit that all antisense methods have is their uniqueness. The difference between targets and non-targets may be manipulated via the specificity of Watson-Crick base-pairing interactions and the almost limitless target selection. In the simplest instance, designing antisense medicines requires just the mRNA sequence. In principle, antisense-mediated suppression should be

effective against all disease-associated genes. The limitless potential of RNAi has enabled the approach of silencing virtually all genes encoding annotated proteins in the human genome on a wide scale. These studies provide critical information to our understanding of genetic function and pathway analysis. With sufficient specificity, target and normal alleles may even be used to replace several disease-specific alleles with only one or a few nucleotides. This is unquestionably advantageous for identifying dominant mutants, such as some oncogenes. RNAi is a more effective antisense strategy than previous antisense methods (such as antisense DNA oligonucleotides and ribozymes) (Bertrand et al., 2002). It is worth mentioning that single-stranded short antisense RNA may also be loaded into RISC and used to guide mRNA cleavage, although the efficiency will be significantly decreased (Martinez et al., 2002). Notably, since RNAi is more potent than antisense oligonucleotides or ribozymes, effector molecules may operate at much lower doses. This is a critical component of the therapeutic environment. The effectiveness of a single target site (often expressed as the half-maximal inhibitory concentration, or IC50 value) varies significantly across siRNAs. The ultimate thermodynamic stability of siRNA is a critical criterion for its effectiveness (Fomenko et al., 1996), accessibility of target mRNA, structural features and other location-specific determinants. Till now, the most critical criterion for siRNA design has been the ultimate differential stability (or skewness), which is compatible with reported miRNA strand selection (Khvorova et al., 2003). Nevertheless, it is far from complete to consider our understanding of siRNA and design target selection. The identification of "super-functional" siRNAs that are functional at sub-nano molar levels is still an elusive problem.

Mechanism activities & Difficulties

RNAi Machinery

RNAi is believed to be a method for gene silencing based on the kind of RNA used. Among them are small interfering RNAs (siRNAs), , short hairpin RNAs (shRNA) and microRNAs (miRNAs) (Z. Wang et al., 2011). The small RNA is integrated in the RNA-induced silencing complex (RISC), which subsequently reduces gene expression by breaking down the mRNA or impeding the translation (Martinez et al., 2002; Zamore et al., 2000).

Argonaut (AGO) is a very important protein for the RISC. There are 8 AGO proteins in humans (Hutvagner & Simard, 2008). Not all of these proteins can cause cleavage. Only AGO2 can carry out the siRNA-induced effects. The protein has three important domains, of which only one of them has RNAase H folding and has "slicer" activity to slice the desired RNA substrates (Hutvagner & Simard, 2008; Liu et al., 2004).

siRNA has defined structure, which consist of short double-stranded RNA with a phosphorylated 5 'end and a hydroxylated 3' end. Typically, it this type of RNA that gets incorporated into RISC directly, this is where the guide chain meets and slices the matching mRNA. After slicing the mRNA is released and the guide strand connects to RISC to bind to another mRNA and cycle begins again (Elbashir et al., 2001; Robb et al., 2005).

Due of siRNA's short half-life, it has been developed as a substitute for the RNA molecule. Typically, shRNA is produced inside the nucleus using an external expression vector containing a short double-stranded DNA sequence with a hairpin loop that is transcribed by RNA polymerase. Then it is treated with an RNase III endonuclease. The resulting product is transported to the cytoplasm, where it is processed by another RNase III enzyme and ultimately integrated into RISC, where it undergoes the same cytoplasmic RNAi process as siRNA.

Furthermore, shRNA is constantly produced by the host cell, it has longer gene silencing effect compared with siRNA. Furthermore, the expense of shRNA is lesser than the mass production of other expression vectors.

As far as miRNA is concerned, it is a small endogenic non-coding RNA that plays a significant role in the management of pathological and physiological cell functions. The miRNA is initially transcribed as a primary transcript in the nucleus. Distinct from shRNA and siRNA, the miRNA is only partly complementary to the untranslated region 3 of the target mRNA, and its target is regulated by four AGO proteins (John et al., 2004). Since a partial match is sufficient for miRNA to function, so changing the expression of miRNA can affect hundreds of genes (Aagaard & Rossi, 2007).

Difficulties

Off-Target Effects

RNAi is thought to be gene selective, with nothing but the genes with matching sequences being impacted. RNAi can occasionally result in non-specific off-target consequences. The miRNA-like binding at the 3' UTRS is a substantial contributor. SiRNA redundancy or chemical modification can help to reduce the unwanted transcripts by complementarity in their 3' UTRs' "seed region" (Birmingham et al., 2006; Jackson, Burchard, Schelter, et al., 2006). From the nucleus to the cytoplasm, the transporter XPO5 transports pre-miRNA or exogenous shRNA. When the RNAi pathway is saturated with shRNA products, miRNAs entering the normal pathway become less accessible. Several discovered components, such as Exportin-5, are involved in the saturation mechanism (XPOS), which is a unique non-specific impact of RNAi expression (Z. Wang et al., 2011).

Delivery Problems

The main limitation in the development of RNAi treatments is the delivery of these macromolecules to target cells. Due of their negative charge and huge size, siRNAs have a difficult time passing through the cellular membrane (Aagaard & Rossi, 2007). Endocytosis is the main cellular entry site for siRNA distribution that is not viral in nature. Upon entering the cells, small interfering RNAs are quickly transported to the endocytotic vesicles (EVs). Initially, they are caught in early endosomes, where the pH lowers to 6, before being transported to the late endosome, where the pH is acidified to 5-6 degrees Celsius. siRNAs that aren't even discharged from such acidic vesicles are ultimately destroyed by the enzymes in the environment (Zhang & Mallapragada, 2011). As a result, siRNA delivery strategies that rely on endocytosis must account for endosomal escape.

By interacting with RNA-binding proteins such as Toll-like receptors (TLRs) and protein kinase receptors, double-stranded RNA activates the innate immune system (PKR) Interferon type I (IFN) and cytokines such as IL-6 and TNFQ are the primary mediators of the inborn immune response. Certain shRNAs or siRNAs may bind and trigger TLR7 if they contain the 5'-GUCCUCAA-3' motif and similar GU-rich sequences, and thus may sequentially stimulate the immune system (Hornung et al., 2005).

Low Bioavailability & Instability

Exposing unmodified siRNAs to high temperature or long exposure at low temperature does no affect their stability (Hickerson et al., 2008). Though RNAi delivery becomes more complex when degradation of nuclease occurs in nucleic acid in systemic delivery. Another study also displays that the accumulated siRNA is excreted to urine from the kidney in 1 hr because of its small size which can become a huge problem (Alexis et al., 2008; Guo et al., 2010).

Chapter 3

Several RNA Drugs and their Bioactivities

Antisense Oligonucleotides

The single-stranded oligonucleotide that specifically inhibits the targeted gene expression was originally described in 1978. The notoriety that ASOs earned through the study of gene functions and new treatments led to an explosion in their use for academic research purposes. Because the natural RNAs are found in nearly all species, a general understanding of their roles in post-transcriptional gene regulation is now well accepted (A. A. Levin, 2019). The suppression of protein synthesis by RNase-independent means, the cleavage of precursor mRNAs (pre-mRNAs) by RNase H and RNase P, and the cleavage of mRNAs by RNase-dependent means are all important in the suppression of target gene expression by ASOs. It can also be used to modulate RNA splicing to build functional proteins or preferred genetic products (McClorey et al., 2006). Furthermore, due to the abundance of RNases H and P in the nucleus, the ASOs are found to be more effective than other RNAs to knock down nuclear targets. The 1st ASO drug approved by the FDA in 1998 is famivirsen, the antisense oligodeoxynucleotide with phosphorothioate linkages which are used for the treatment of cytomegalovirus retinitis. In the United States, there are several ASOs therapeutics marketed successfully among which the gapmers are mentionable which contains the modified antisense oligoribonucleotides at both 5′ and 3′ ends with a “gap” of oligodeoxynucleotide in the middle (Aartsma-Rus, 2017). Researches are being made for the new ASOs for the cure of cancers, infectious diseases and genetic disorders. To illustrate, ASO therapy can be used for the remedy of inherited retinal dystrophy, a deep intronic c.2991+1655A>G mutation in CEP290 underlying Leber congenital amaurosis type 10 (Dulla et al., 2018).

Small Interfering RNAs

The RNA interference development and discovery have advanced the technology with double-stranded RNAs (dsRNAs). The siRNAs with 18 to 22- b have already entered clinical drug development and are regularly used for the selectiveness and effectiveness of the knockdown of the target gene expression in basic research (Castanotto & Rossi, 2009). The guide strand of the siRNA is distinguished from the other two strands by the presence of two 3′-overhang ribonucleotides, which are essential for the duration of gene silencing. This process is carried out by the endoribonuclease Dicer or helicase with RNase motif and guide. The RISC is responsible for detaching the passenger-strand of the dsRNA after trimming it. The endonuclease argonaute-2 is the catalytic core of RISC which cleaves the passenger strand. The RISC has to hold on to the 5 ends of the guide strand which is thermodynamically less stable. It leads to a sequence-specific cleavage of the targeted mRNA by argonaute-2 and consequently the knockdown of genes (Lennox & Behlke, 2016). The advancement of siRNAs needs to attain potent, specific, and long-lasting gene silencing while minimizing off-target genes.

The RNAi mechanism has been studying for years and has a better understanding which provides some guidelines to be followed and usage of the specific software to design the effective siRNA. The effectiveness of the siRNA depends on the proper target site which is closer to the start codon within the coding sequence. Another important part is to ensure selectivity and lessen off-target effects of siRNA. The stability and efficacy of siRNA are affected by its composition like the overall C/G content and use of specific ribonucleotides at particular locations as this can be induced the immune system into sequence-dependent and sequence-independent manners. Moreover, it is very essential to avoid immune-stimulatory motifs for designing siRNAs. They are also introduced into other therapeutic fields like

oncology which benefits thousands of patients. To demonstrate, the siRNA siG12D-LODER precisely targets the mutant Kirsten rat sarcoma virus oncogene (KRAS) G12D mRNA, a driving oncogene seen in a variety of malignancies, most notably pancreatic cancer (Khvalevsky et al., 2013).

In a clinical study of phase 1 study is it observed that the treatment given with siG12D-LODE and gemcitabine to a pancreatic cancer patient was well tolerated and developed potential efficacy. Similarly, siRNA is found to be effective for controlling the growth of tumors in xenograft mouse models by treating a protein tyrosine kinase name ephrin type-A receptor 2 (EphA2) (Wagner et al., 2017).

RNA Aptamers

Specificity and high specificities are two important characteristics of single-stranded DNA or RNA oligonucleotides that bind to a wide range of molecular features. These include DNAs, RNAs, peptides, small molecules, proteins, and ions that have specificity and high specificity. To control the disease RNA aptamer acts like a chemical inhibitor or nucleic antibody for modulating protein function when they are binding to the proteins. The natural RNA-protein complex was priory introduced in bacteria where in the activity of the RNase P complex to the process of turning the precursor tRNA into active TRNA, the RNA molecule is the fundamental element. There are many RNAs or aptamers which could inhibit this process. When the autocatalytic RNA or ribozyme binds with the monovalent and divalent cations they naturally undergo self-splicing. In addition, the intrinsic RNAs or riboswitches can control the target gene expression by sensing the small molecule metabolites. Moreover, in humans, the presence of ligand-binding ribozymes and riboswitches has been observed in research (Ray et al., 2009).

As a result of studying RNAs, we have gained a better knowledge of the interactions of functional RNA with ligands, as well as the interactions with a range of proteins in the

environment. Again, comprehensive evolution of ligands by exponentially enrichment was established to produce highly selective and powerful RNA aptamers or ribozymes and to detect them using a high throughput technique. Furthermore, same as the ASOs and siRNAs, the PK properties can be improved and the metabolic stability can be increased through the chemical modifications of the selected RNA aptamers. Spiegelmer, an artificial aptamers can be developed and synthesized by the mirror-image L-ribonucleic acids which is resistant to the degradation by RNases (Vater & Klussmann, 2015). In 2004, the FDA approved Pegaptanib, the first RNA aptamer medication, to treat neovascular are associated with muscular degeneration (AMD), demonstrating the potential of aptamers to interfere on target proteins in the treatment of human illnesses. Olaptesed pegol (NOX-A12), a 45-nt RNA Spiegelmer, has been developed to specifically target the small chemokine stromal cell-derived factor 1 or the C-X-C motif chemokine 12 with high affinity. Olaptesed pegol (NOX-A12) is a pegylated 45-nt RNA Spiegelmer that has been designed to selectively target the small chemokine stromal cell-derived factor 1 or the C motif. The function of this drug is to prevent the binding of the stromal cell that is obtained from factor 1 to its receptors CXC receptor 4 and CXC receptor 7 which actively inhibits the subsequent signal transduction. To control the angiogenesis and metastasis the signal transduction has been inhibited along with the improvement of the other anticancer therapies (Roccaro et al., 2014).

Messenger RNAs

Messenger RNA is a novel class of drug modalities, it has an incredible role in the immunization process like vaccination, antibody therapy, and protein replacement therapy. In 1990 it was first confirmed through the findings on the efficient expression of target proteins in the mouse tissues in vivo which was later of the administration of in vitro–transcribed (IVT) mRNAs. There are many IVT mRNA therapeutics which has entered clinical trials by

undergoing extensive preclinical studies (Grogan et al., 2012; Papachristofilou et al., 2019). In contrast, virus-based gene therapy or plasmid virus drugs of mRNA do not interfere with the genome but are translated into target proteins by cellular machinery. In addition, the mRNAs have to have the whole open reading frame of a target protein and intact 5′ and 3′UTRs as well as 5′ cap and 3′ poly *(*A) tail to make sure the efficiency and translation ability and this made the mRNA drug molecule bigger to all other types of RNA therapeutics. Similarly, the mRNAs need to be protected from the degradation of RNases and cross cellular barrier when it is directly administered to the patients and also the mRNAs that are preprogrammed with dendritic cells or autologous transplantation of T cells can be used to treat the penitents.

The mRNA therapeutics are widely used as vaccines for cancerous and infectious diseases for their high sensitivity for recognizing the antigens of immune cells wherein the exogenous mRNAs can play the role in coding. To illustrate, the autologous transplant of DCs is transfected with mRNA encoding prostate-specific antigen to induce the prostate-specific antigen-specific immunity and to have an impact on surrogate clinical endpoints (Fotin-Mleczek et al., 2011).

Likewise, to achieve gene editing mRNAs are used to introduce the target proteins which includes usage of mRNAs as transcription activator-like effector nucleases, transposes, CRISPR-associated proteins, or endonucleases (e.g., Cas9 and Cas12a) and encoding zinc finer nucleases. By so far the safety and specificity of gene editing it has reached the preclinical investigations as this needs much more critical and extensive studies (Gurumurthy et al., 2019).

Guide RNAs

In terms of editing the genome sequence precisely the prokaryotic CRISPR Cas immune system has been used to knock in or knockout a target gene. When mRNA is differentiated with the

RNAi is it observed that RNAi doesn't fully reduce the expression of genes but mRNA therapy can quickly produce proteins. There are two most important components needed for the CRISPR/Cas gene-editing technology which are RNA-guided Cas nuclease and a designed gRNA. The guide picks up the protospacer-adjacent motif element using its hairpin scaffold. Subsequently, it binds to Cas making a ribonucleoprotein complex (Cas-gRNA). It instructs the nucleus of Cas to produce a DNA break to edit the genome. To treat diseases such as cancer, monogenetic disorders, or infections the technology is being evaluated towards the development of new therapies (Georgiadis et al., 2018).

The mechanism of gRNA is completely different from the other types of RNAs as here the exogenous gRNA is combined with the foreign Cas nucleus to succeed the CRISPR/Cas genome editing and therapy which turns into a bi protein of size 160 kDa later. To perform the genome editing the gRNA and Cas protein has to be induced in the nucleus to form RNP. Moreover, the gRNA can be produced by chemical synthesizing or IVT and directly introduced into organisms and cells with the specific delivery system by using virus vector-based or plasmid DNA materials, sometimes it can combine with the Cas nuclease as RNP. Though there are researches taken out to ensure the safety and effectiveness of CRISPR/Cas-based therapies there has been no satisfying result obtained. In 2016 the first clinical trial for CRISPR/Cas gene editing was used to treat the patient with NSCLC by knockout T cells with the help of PD-1 (Program cell death protein 1). The T cell activation is inhibited by the membrane receptor PD-1 immune checkpoint regulator which decreases the autoimmune reactions and allows the immune escape of cancers. To treat different types of cancers the antibodies against PD-1 or the ligand of it are used successfully and for the combat cancer, CRISPR/Cas based PD-1 immunotherapy represents a novel strategy. Furthermore, in 2018 in the phase-I trial, the safety profile of NY-ESO-1 is defined by redirecting the autologous T

cells with CRISPR edited endogenous T cell receptor (TCR) and PD-1 autologous T cells. The patients with severe sickle cell disease and transfusion-dependent β-thalassemia are treated with the autologous CD34+ hematopoietic stem and progenitor cells that were edited using CRISPR/Cas. Similarly, for the pharmacotherapy of relapsed B cell malignancies, the CD19-directed T cell immunotherapy with T cells altered CRISPR/Cas is undergoing clinical evaluation to determine the efficacy and safety (Sahel et al., 2019).

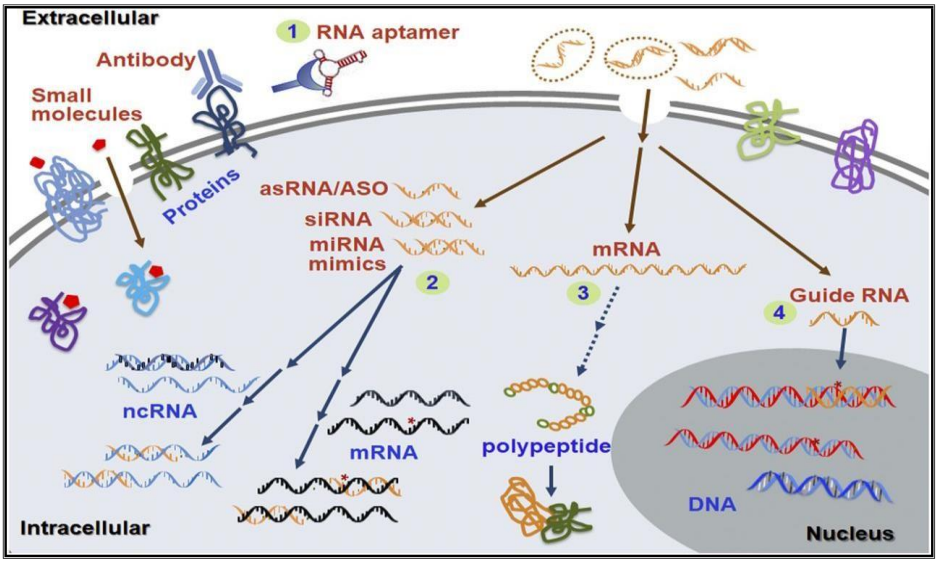

Figure 4: Illustration of the favorable targets for small molecules, proteins, RNA aptamers (Damase et al., 2021).

Chapter 4

RNAs as Therapeutic Drugs & Challenges

Applications of RNAs

Genetic Diseases

To begin with, researchers are investigating the use of RNA interference to treat genetic disorders. Studies showing that SNP in dysregulated allele transcripts may be exploited as selective targets for RNAi offer a potential lead in this area. Because CAG repeats are prevalent

in many normal transcripts, it is difficult to specifically target them using short interfering RNA. Mutations in the coding region of the gene may frequently result in single nucleotide polymorphisms (SNPs), which offer potential selection targets (Miller et al., 2004). Our goal is to develop a highly selective siRNA/SNP combo. The method that has been used to synthesize siRNAs that have distinct nucleotide sequences in the middle is a step-by-step evaluation of the siRNAs to search for differing nucleotide sequences. Using siRNAs, only the mutant transcripts are selectively degraded, while the wild type transcripts are left intact even if just a single mismatch exists with the wild-type sequence. The use of an extra siRNA targeting a single nucleotide polymorphism (SNP) was discovered (Ding et al., 2003) in research on the common genetic condition ALS, which is responsible due to the mutation of Zn, Cu superoxide dismutase gene (SOD1). When Schwarz et al. recently checked out the properties of the SOD1 and the huntingtin (HTT) gene variants, they discovered that the siRNAs they used discriminated between wild-type and mutant alleles. They discovered that SNPs might produce mutant-specific siRNAs even if mismatches are positioned sensibly. At nucleotide positions 10 and 16 relative to the 5 'end of the guide strand, purine–purine mismatches give selective sensitivity (Ding et al., 2003). Since wild-type SOD1 serves essential activities, only the mutant allelic transcript should be downregulated. Single nucleotide alterations observe in many SOD1 mutations. With this investigation, researchers were able to degrade a mutant allele expressing SOD1, a possible therapy for ALS. The use of RNA interference (RNAi) to cure degenerative and neurological illnesses may approach reality very quickly since it is possible to transport the siRNAs and viral vectors that express them to the afflicted areas of the brain (Miller et al., 2004)

Viral Diseases

In vivo, RNA interference was shown to be effective when the HBV replicon and the pol III RNA expression unit were co-delivered via a hydrodynamic method in mice (Z. Wang et al., 2011). Previous research showed that it was possible to reduce the HBV core antigen to 99% of its normal levels in hepatocytes by using shRNA, proving to be a valuable use of RNAi for HBV antiviral purposes in the liver. HCV is the primary etiology of liver transplants in the United States, as well as a common reason behind chronic liver disease and liver cirrhosis, both of which lead to the development of liver cirrhosis and hepatocellular carcinoma. Currently, the sole treatment on the market utilizes IFN and ribavirin in combination. While progress has been attained in the treatment regimens, it is uncommon for patients to respond, and specific HCV subtypes seem to be exceptions. Perhaps because the life cycle of HIV was well known, as was its pattern of gene expression, the first infectious pathogen addressed by RNAi was HIV. Also known as synthetic and expressed siRNAs, siRNAs such as the TAR elements have been utilized to target HIV early and late RNAs, including the elements of TAR like Gag, and reverse transcriptase (Novina et al., 2002). HIV replication inhibition is also possible via RNAi of cellular cofactors, such as NF (which is also known as NFKBIB), HIV receptor CD4 (which is also known as CEA), and co-receptors CXCR4 and CCR5 (which are also known as CXCR4 and CCR5). Furthermore, across a wide range of human cell lines and primary cells, suppression of HIV replication has been accomplished. There is a strong likelihood that RNAi therapy will make it to the clinic within the next year or two because RNAi is much more potent than ribozyme or antisense methods (Amado et al., 2004).

Cancer Diseases

This new approach to treating cancer may completely change the way we treat it. The research difficulties for cancer are comparable to those found for other illnesses. They include the search

for appropriate targets, the use of delivery methods, and efforts to minimize toxicity. So far too many targets for oncology have been observed in the literature. While there were many methods developed to employ transferrin-loaded nanoparticles. This is perhaps the most important study using this method due to its potential clinical benefit. This research shows that non-lipid-based nanoparticles may effectively be used to deliver siRNAs to metastatic malignancy. In many studies, the use of mouse xenograph models is beneficial in reducing tumour development (Takeshita & Ochiya, 2006). Some examples of recent advances in treatment include the use of adenoviral or retroviral delivery of shRNAs that target Hec1, which resulted in better tumour growth inhibition in patients with adenocarcinoma-induced tumour growth, aptamer-siRNA chimeric RNAs that successfully targeted prostate cancer cells by aptamer binding to surface-expressed tumour cell marker, and atelocollagen complexed siRNA that inhibited Vascular endo. The newest class of targets to consider is miRNA since this offers several still untapped therapeutic options for the treatment of cancer (Hammond, 2006). MicroRNAs are known to play significant roles in cell differentiation. They have also been proposed to act as oncogenes (oncogenes are tumor-causing genes) or tumour suppressors (tumour suppressors help combat the development of tumors). Antisense-based mechanisms allow for down-modulation of target transcripts, and expression in the endoplasmic reticulum (ER) rescues deregulated miRNAs as demonstrated in *C. elegans* (Johnson et al., 2005). It's also possible that RNAi may be used to turn off pathways that let conventional cancer treatments do their work. In this context, we are talking about: first, treatment of cancer cells that possess a multidrug resistance gene (MDR1) by re-sensitizing the cancer cells to chemotherapy; secondly, inhibition of double-strand break repair enzymes for more cancer cell killing with radiation and/or chemotherapy.

RNA Currently Used In Chemical Practices

The FDA has authorized a variety of RNA-based medicines for the treatment of different human illnesses. The FDA approved fomivirsen, which is an antisense oligonucleotide (ASO), the treatment of retinitis in HIV-infected individuals who have developed acquired immune deficiency syndrome (AIDS) also done in 1998. At least a half-dozen ASO drugs, several of which have been approved by the FDA in the U.S. since 2013, have been able to successfully be marketed here (Aartsma-Rus, 2017). Other aspects of pegaptanib's approval for use in the therapy of age related macular degeneration (AMD) indicate the likelihood of utilizing an RNA aptamer to specifically target and inhibit protein macromolecules in the treatment of a disease. Another encouraging piece of news in the field of RNAi is the FDA's recent approval of a double-stranded siRNA therapy for patients with hereditary transthyretin amyloidosis (Wood, 2018). This approval is being seen as an impetus to promote the further development of RNAi-based therapies.

Challenges in Developing RNA therapeutics

Contesting with the Endogenous RNAs

Medications that are bioactive, depends on the cellular processing for running the danger of overloading those pathways, disrupting the natural system. Because siRNA/shRNA rely on the natural microRNA system to induce potent target silencing, ectopically injected RNAi triggers operate similarly. Although the discovery of miRNAs has dramatically increased the capability of understanding the RNAi roles in human cells. Nonetheless, investigations of miRNAs show that they play a crucial role in regulating gene activity (Farh et al., 2005). MiRNAs have been implicated as oncogenes or tumour suppressor genes, for example. The early embryonic lethality of Dicer knockouts (Bernstein et al., 2003). It suggests that a functioning RNAi machinery is undoubtedly required for mammalian cells. Because siRNA and shRNA resemble

excessive amounts of ectopic RNA may obstruct all components of the miRNA pathway. It was just discovered in a crucial paper by Mark Kay's lab (Grimm et al., 2006). It is reported that death in mice after high dosages of liver-directed were given. Many shRNAs examined caused lethal condition to the mice in less than two months. Reduced expression of liver-specific miRNA was linked to morbidity.

Competition studies revealed that Exportin 5 improves RNAi efficacy in vivo, implying that liver toxicity may be caused by miRNA pathway saturation. Nuclear export is also a potential rate-limiting step. We have found that employing a combination of the pol III U6 promoters and the pol II U1 promoter to combine low-level expression of the same shRNA results in more effective RNAi than using a single U6 promoter. This is due to U6 transcribed shRNAs saturating the Exportin 5 pathway. Other aspects of the RNAi apparatus may be prone to saturation, necessitating more research using animal model systems. The most essential takeaway from this study is that vector-based siRNA expression systems should use better promoter systems in combine with low-dose siRNA.

Stimulation of Natural Immune Retaliation

The discovery of double-stranded RNA was thought to induce innate RNA that would shut off in mammalian cells. Until Elbashir et al's breakthrough discovery, it was felt the only a matter of time before the cell stopped working. Long-stranded ribosomal RNA (dsRNA) is known to induce both interferon responses by binding to protein kinases and Toll-like receptors. The TLR7 of dendritic cells appears to be identified as the unique sequence motif (5′-GUCCUCAA-3″) and trigger immunological responses. The study shows that certain siRNA sequence motives elicited immunological activation depending on TLR 7 (Hornung et al., 2005). The discovery of the so-called "dangerous motifs," GU-rich areas which have proved themselves to trigger innate immune responders and lead to cell-type and sequence release of inflammatory

cytokines, was published in. Since then, several publications have identified what they call the dangerous motifs - oligonucleotides that indicate the "danger" of hostile nucleic acids on the cell through TLR9 for immune-stimulatory patterns of CpG. The induction of siRNA-mediated immunity seems to rely on TLR receptors (Marques & Williams, 2005), but cells are heavily affected by the way the syRNA is delivered, thereby dividing it. Although understanding of immune stimulation features requires a study on animal models, it is also necessary to in vitro use of primary humans with an extensive repertory for immune stimulation. Recent study showed that induction of cytokine might be repealed with preserving silence activity (Morrissey et al., 2005). Although these potential results are encouraging, significant attention and extensive tests are definitely necessary before clinical application.

iRNAs (Darmacon) have been shown to increase the number of interferon reactions in cell culture tests. Darmacon has recently released a study suggesting that short 23 nt siRNA and interferons may be linked to enhanced immunological activation. The danger of enhanced immune reaction should be carefully considered. iRNAs in the cell types appear to differ according to the length threshold, which makes it difficult to predict the result of Dicer substrate siRNAs. T7-transcribed siRNAs in vivo. Interferon reactions are strongly influenced by the presence of a 5′ interferon reaction (Hornung et al., 2005). The researchers say that previous results should be looked at in additional examination.

Suppression of off Targets

RNAi's almost perfect characteristic did not prove to have been entirely accurate. Genome-wide gene activity monitoring by micro-array technology has convincingly demonstrated that cells treated with siRNA display a high amount of gene silencing. The silencing of off-goals is evident since it is unclear and generally unpredictable how the biological effects of changing gene activity are. In vitro transfected cell studies reveal that a third of siRNA randomly picked

impact the cell's survival and can show that off-targeting leads to a hazardous phenotype (Fedorov et al., 2006). The so-called "seed" region has a match of 6-7 nuclear oils. Study showed that 11 nucleotides matching the target with the siRNA may result in a knock-down off-target. Recent study has made it more evident that targets have been removed. This has been shown in previous studies of the mirRNA-target mRNA pairs. It can be used to identify the mical RNA as a potential target. In target selection, position one of a miRNA is irrelevant the interaction between 5′phosphate and PIWI does not match the mRNA objective. The goal in the 3′ UTR which imitate miRNA bindings are preferably silenced if hexamer regions are included in them (Jackson, Burchard, Schelter, et al., 2006). In this case, it is possible to silence the 3 UTR by removing the PIWI domain. They say this could make it challenging to determine which genes are affected by translation inhibition instead of mRNA instability. But the picture is far from comprehensive in terms of identifying targets because many genes with a strong match of the seed continue to be impacted (Birmingham et al., 2006). It's also likely that the cell targeting will not only have an impact on mRNA but also on miRNA expression patterns. The new technology in protein arrays can give a better opportunity of siRNA impacts on the expression of cell proteins and offer better screening options for SiRNA. Non-targeting remains a crucial challenge in RNAi therapeutic use and siRNAs with acceptable levels require practical testing. Studies showed that researchers have developed a new way to identify siRNAs that can be used to prevent undesired targeting. They found that 2′ O-me modified siRNAs can decrease the targeting substantially without affecting the target Silencing Degree (Jackson, Burchard, Leake, et al., 2006). The intelligent design of the SiRNA that enhances selectivity of the strand should not benefit passenger strand loading which has been displayed cause off targeting.

Differential thermodynamic encourage the incorrect transport of a 5″ beach with energy, which may substantially increase cleavage efficiency and deliberate mismattures in the passenger

beach. Passenger bearings may be easily targeted by constructing fake meaning and antisense targets, such as a commercially available psiCheck vector system. In the case of fixed target sites, improvement of beam loading may be reduced and additional siRNA alterations may be required to enable effective differentiation between normal and mutant transcript. The only 5" end of the guide strand connects with the mRNA targets whereas in target recognitions, the 3" end plays a minimal role (Doench & Sharp, 2004).

Chapter 5

Different Types of RNA Targets & Their Bioactivities

The Ribosome

The new era antibiotics are very much reliable to RNA binding small molecules. The RNA binding drugs shows their effectiveness by binding with the rRNA. The rRNA and ribosomal proteins are the building block of the two subunit, large (50S) and small (30S). The large subunit (50S) is in care of processing of peptide bond formation whereas small (30S) subunit is held accountable for incorporation the only correct tRNA into the amino acid. The rRNA is only behind the activities carried out by the subunit which are, catalysis by the 50S subunit and proof reading by 30S subunit. In the 16S rRNA which is the part of 30S subunit the proofreading occurs. For adopting an extrahelical conformation the tRNA induces the residue A1492 and A1493 (Hermann, 2003).

Aminoglycosides are the common RNA-binding small molecules which has been ardently investigated among all the other classes of antibiotics which binds to the 30S subunit. Aminoglycosides binds with the A site to incorporate the amino acids from noncognate tRNAs by binding with the 4, 5- and 4, 6-substituted. Moreover, aminoglycosides allows the residues

A1492 and A1493 to create as same as the binding cognate tRNA from the helix (Ogle et al., 2001).

In addition, combining with linezolid, oxazolidinones the newest class of antibiotics which has entered to the clinic as the first of this class. The mechanism of oxazolidinones is to bind with the 23S rRNA that is present within the 50S subunit. To illustrate, to bind with the 50S subunit oxazolidinones is observed. In the translation assays to isolate ribosomes with weak affinity the oxazolidinones binds in spite their low micro molar IC50 values. Furthermore, the initiator tRNA is prevented from binding with the 50S ribosome by the oxazolidinones. So it is understand by this mechanism that to prevention of peptide bond formation at first, oxazolidinones binds near the P site as the binding affinity for ribosomal subunits are observed (Jarvest et al., 2002).

Transfer RNA

Aminoacyl-tRNA synthetases (AaRs) has gained huge interest in the antibacterial field for its inhibition characteristics. It is an important enzyme which works for coupling of the amino acids to give rise to tRNAs. The inhibition of the AaRs has been recognized as an effective antibacterial strategy for leading the uncharged tRNA to inhibit the protein synthesis and finally death of the bacterial cell. The AaRs or the tRNA are targeted for preventing the inhibition of the aminoacylation reaction by interacting the AaRs with the respective tRNAs. There are many aminoglycosides found to bind with tRNAPhe where the neomycin is found (Figure 6A), also the neomycin was collaterally effective to inhibit the charging of tRNAPhe, *K*i of ~300 μM.63. Besides, to inhibit other compounds like non-aminoglycoside small molecules are found to be effective though the concentrations of the ligand required to perform this function is excessively high. In the variety loop region there are nucleotides which make the tRNAPhe, a binding site for neomycin which states that the specificity for tRNAs could be achieved by

the rationally targeting loops which are located on the distant tRNAs (Kirk & Tor, 1999; Walter et al., 2002).

Riboswitch RNA

By recognizing the small molecule metabolite, riboswitch RNAs regulate the protein translation of the post transcription. The riboswitch RNAs are the type of mRNA that is buildup with two domains which are obtained at the 5′-UTR of an mRNA primarily. This is a domain which is tied up to the ligand with high affinity and specificity and the other is a platform domain which is to hold to conformational change to retaliate the ligand binding and modulates translation. The riboswitches are abundantly found in fungi, bacteria, plants and archaea and it is advised in a study of bioinformatics that mRNAs (Mandal et al., 2003).

By replacing riboswitch by a different aptamer domain, the ligand mediated translational control of the riboswitch is modulated. This kind of adaptability helps it to engineer the artificial riboswitches in functions like sensors and control of bacterial mirastion. Similarly, it has been determined that there are many riboswitch aptamers with different structures which is bound with their cognate ligands and many secondary structures are formed as the bending site of each ligand is formed. To differentiate the cognate and near cognate regions the binding pocket of each molecules create a complex array of contact such as, when elimination of a single methylene unit from the methionine side chain the riboswitch exhibits decrease in affinity by 75-fold (Winkler et al., 2004).

In the previously determined solutions of confirmations the metabolites that are bound with ligand conformations were nearly identical in their respective binding pockets. This function of the targeted riboswitch RNAs which helps to regulate important genes by using the small molecules whichever mimics metabolite of the riboswitch was proposed by Beaker and Blount. This can be a novel antibacterial agent as the metabolite analogs pyrithiamine pyrophosphate,

aminoethyl cysteine, L-4-oxalysine, and roseoflavin which shows a characteristic of exhibiting antibacterial properties can be effective by targeting the particular riboswitch RNAs. The mutation in the aptamer domain of the each of these metabolite mimics alters the riboswitch ligand interaction. Lastly there are undergoing researches taken out to identify riboswitch inhibitors by high-throughput screening and assaying structural mimics of metabolites (Blount & Breaker, 2006; Mayer & Famulok, 2006).

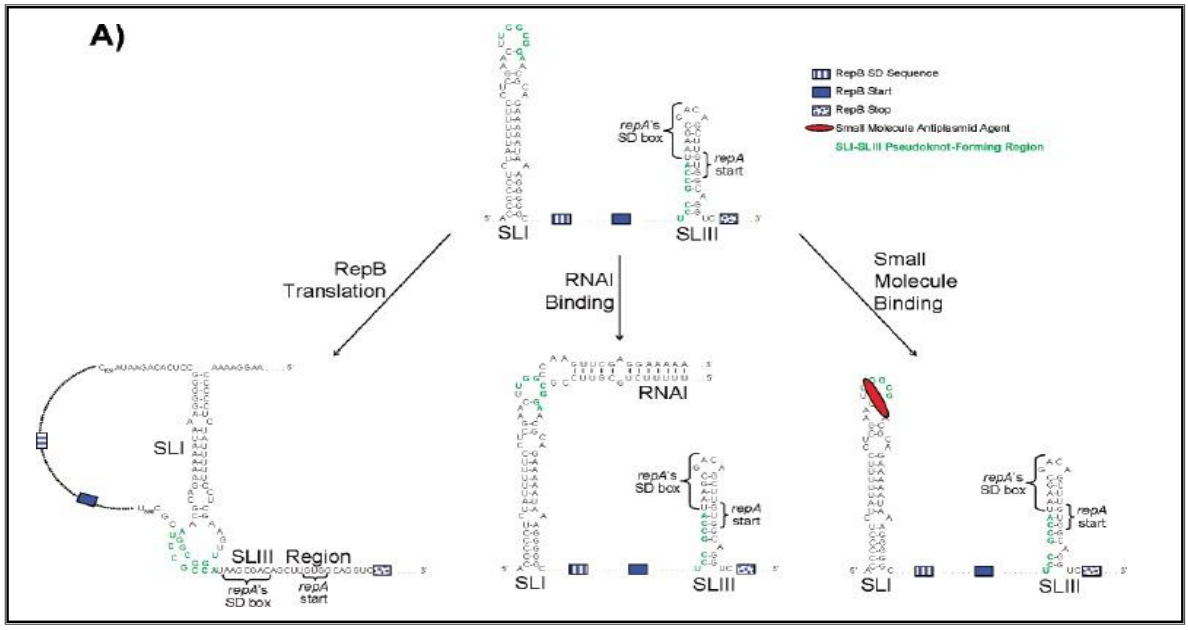

Figure 5: Translational control in the IncB plasmid replication system that is Antisense-mediated. (Thomas et al., 2005)

Antisense Mediated Translational Control

The antisense mediated translational topic is very common in bacteria and prevalent in plasmid replication systems. The mRNA translation in the post transcriptional control system is controlled by the complementary RNA. Moreover, in the determined mRNA the translational state of the mRNA is regulated by the antisense RNAs by binding with the base pair. In the study of the bioinformatics, it is observed that the hairpin loops which is involved in the RNA-

RNA interactions generally includes a YUNR sequence where “Y” is a pyrimidine, “N” any nucleotide, and “R” purine, which is known to form a U-turn motif. To boot, the structural motif helps the hairpin to adopt in the phosphate backbone of sharp bend by forming the bases in the loop in such a way like Watson-Crick base paring (Judy Praszkier & Pittard, 2005).

This sort of RNA-RNA interaction can make a scope for the improvement of antiplasmid agents or novel antibiotics. Moreover, it has recently came to acknowledgment that antiplasmid agents in the IncB plasmid system can work as as here the plasmid copy number are controlled by the RepA protein which is a phosphodiesterase to start the replication of the plasmid. One of the antisense mechanisms is responsible for controlling the RepA mRNA stringent translation. Furthermore, multiple stem-loop structures are formed. The combination of stem-loop structure (SLI) with the antisense RNA that prohibits the translation since the Shine-Dalgarno (SD) sequence is paired with the base in the duplex region of a stem-loop (SLIII), having said that the free SLI binds with the SLIII for stabilizing the open form of the helix and to exposing the SD sequence which allows the translation of the RepA protein and leads to the plasmid replication in the end. It was earlier found that they prevent the plasmid replication and leading to the plasmid loss the association with the SLIII is inhibited by the small molecules which can mimic the function of the RNAI by binding with the SLI (Thomas et al., 2005).

In the clinics the plasmid removal strategy can be useful as bacteria generally harbor multidrug resistance plasmids because the removal of the plasmids would make the bacteria susceptible to antibiotics to which they were formerly been resistant. Additionally, a group of aminoglycosides was screened to check their potentiality to bind with SLI for identifying the potential antiplasmid agents. SLI was bound with all the 4, 5- and 4, 6-deoxystreptamine aminoglycosides with mild-nanomolar affinity in contrast to the hygromycin B and spectinomycin which shows no binding affinity. Besides, apramycin can induce the loss of

plasmid in the dose and time dependent manner which is obtained from the bacterial cell cultures results. It binds with the SLI binding bacteria to exhibit the antiplasmid effect as apramycin cannot eliminate the SLI mutant sequence in the plasmid (DeNap et al., 2004).

Protein Mediated Translational Control

Proteins can be used in the mammalian cells can be considered as analogous to small metabolite molecule and the mechanism of the antisense mediated translational control such as, binding with the own mRNA, the thymidylate synthase (TS) negatively regulates its expression. In two distinct site of the mRNA the TS is bound where the site 1 is resided in the first nucleotides that contains coding portions and the 5′-UTR portions and the site 2 is found further downstream in the coding regions. The binding site for the TS has a six-membered hairpin loop and two small symmetric internal loops which is called as 35 nucleotide stem-loop structure (J. Praszkier & Pittard, 2002).

Again, the start codon for the TS mRNA in the hairpin loop resides has the AUG sequence which is important for the binding of the TS because the corresponding AAA mutant could not bind with the TS. Moreover, the attraction of the pharmaceutical R&D programs through many years. For the synthesis of the thymine monophosphate TS has the only responsible enzyme, then to incorporate into DNA it is again metabolized into thymine triphosphate. On the contrary, TS fails to regulate its own translation as the certain classes of inhibitor makes it ineffective (Chu & Allegra, 1993). The TS inhibitors has less effectiveness when the concentration of TS is increased in the cell so an alternate approach in needed for the inhibition of the activity of the enzyme. Furthermore, in low iron conditions the regulation of ferritin and transferrin receptors are found to work in synergy because the iron is imported by upregulation

transferrin from the surrounding environment into the cytoplasm and also the protein prevented from binding and sequencing iron by the simultaneous down regulation of ferritin.

Iron regulating proteins 1 and 2 regulated the levels of the receptors of ferritin and transferrin where IRP-1 inhibits the translation by binding with the ferritin IRE the by preventing the initiation factors to bind with the 5′-cap of the ferritin mRNA. The new researches on the bioinformatics have described about the sequence and the structure of the RNA segment which makes the IREs highly conserved in secondary structure. When the IRP-1 binds the bulged region ensures the change on the molecule by acting as a molecule hinge and if this bulged region is deleted the binging affinity is decreased to nearly 400-fold. For the treatment of the sickle cell disease the availability of the iron modulation by the small regulation molecules (García, 2018).

Chapter 6

RNA as Therapeutic Targets for Small Molecule & challenges

Approaches to Affect Small Molecules

Informa's base is in querying these highly probable regions of structured RNA elements against empirically established. Several ways for rationally designing chemicals that intentionally target RNA and alter downstream biology are constructed into Informa. Because selectivity is still a significant challenge in small molecule RNA targeting (Costales et al., 2017). Furthermore, these RNA 3D folds can be restricted to bioactive portions of RNA. Small compounds that have been targeting the precursors of oncogenic miRNA have been created using Informa's highest fitness and most selective binders, and have demonstrated encouraging preclinical findings in vivo (Huang et al., 2009).

According to research, Inforna defined interactions between RNA-small molecules can be used to guide the selective targeting of miRNA precursors. The carcinogenic miR 210 is an excellent example of this. MiR-210 expression is abnormal in tumor cells that are exposed to hypoxic conditions, such as those found in solid breast cancer tumors. Targapremir-210 (TGP-210), a drug discovered by Inforna, suppresses miR-210 production by binding to the C/C internal loop exhibited in its Dicer processing site. 80 In cellular and in vivo models, inhibiting miR-210 disturbed (Kelly et al., 2011).

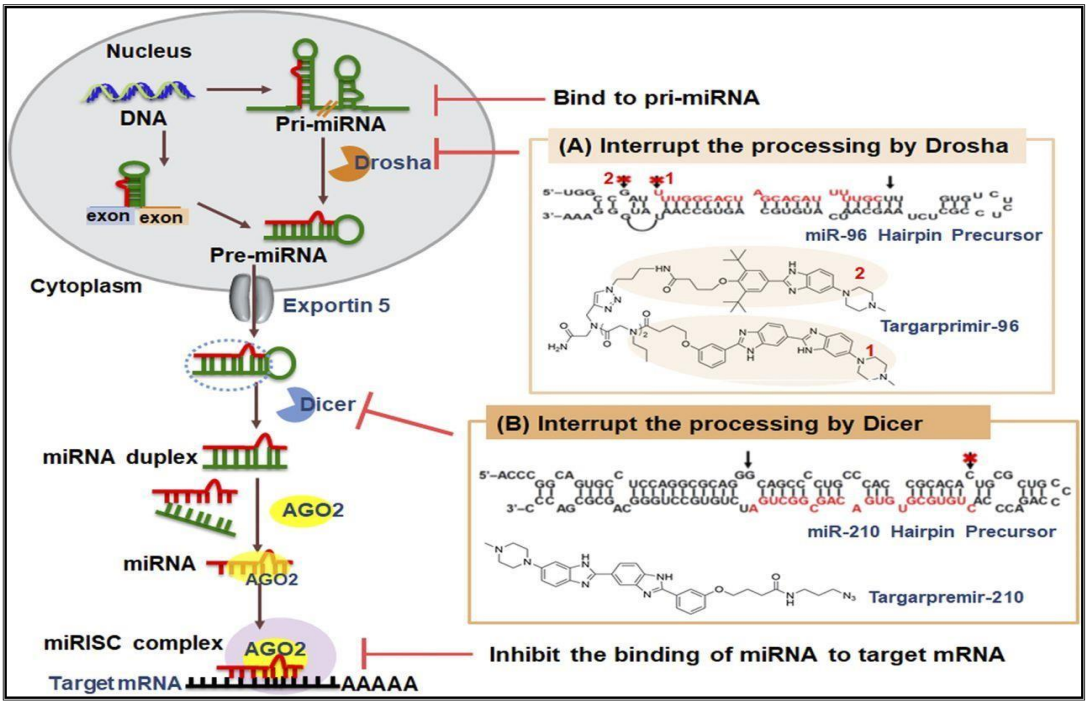

Figure 6: Pri- and pre-miRNAs as therapeutic targets for small-molecule compounds.(Yu et al., 2020)

Quantification of Selectivity

Quantitative analysis of compound specificity with kinase inhibitors was previously measured. A particular measurement system called Gini coefficient, originally developed as a statistical distribution measure of the distribution of wealth to measure inequality (Bosc et al., 2017). The Gini coefficient was recently used to quantify the selectivity of kinase inhibitors because it

indicates a frequency distribution of distinguished inhibitions between the kinase populations. Briefly, the Gini coefficients range between 0, which indicates a non-selective compound, and 1, which indicates single target selectivity. A kinase inhibitor that has no selectivity (testing 78/85 kinases), for example, has a Gini coefficient of 0.15 while selective inhibitors have a Gini coefficient of 0.69 to 0.9 (Drewry et al., 2017).

Similar selectors of a tiny molecule affecting pri-miR-515 and Vivo-Morpholino modified ASO targeting miR-515-5p have been applied to RT QPCR profiling data from MCF-7 cells in this analysis with respective Gini coefficients are 0.75 and 0.72. A comparable level of selectivity with Gini coefficient values 0.71 and 0.62 showed respectively in the smaller molecule and ASO targeting miR-96 in other analyses. In addition to qPCR data profiling, Gini coefficient analyses for large datasets can also be applied. For instance, the large selectivity of a small molecule targeting miR-544 (Gini coefficient = 0.73) and a miR-544 ASO targeting (Gini coefficient =0,70) was shown in transcriptome-wide microarray analysis. Surprisingly, the monomeric miR-544 has high drug-like features that support the idea that the traditional 'drug-like chemical space is also occupied with selective little molecules that target structured RNA patterns. In general, metrics such as the Gini coefficient are useful tools for comparing compound selectivity, to objectively assess the selectivity of small molecules interacting with RNA. Notably, the selectivity of the small molecules is rivals or exceed that of ASOs by Gini coefficient analyses (Haga, Christopher L., Velagapudi, S.P., Strivelli, J.R., Yang, W.-Y., Disney, M.D., Phinney, 2015).

Targeting Viral RNA Sequences

While aminoglycoside antibiotics preferably bind RNA to DNA and remain a valuable leading force to develop new RNA binders, they have 2 important shortcomings- moderate affinity and inadequate specificity. Their promiscuous linkage to many RNA objectives gives rise to

concerns about their potential usefulness. We felt that the empirical knowledge base of RNA and ligand interactions had to be substantially expanded. Efficient means for the quick screening of chemical compounds have become necessary for their RNA affinity and selectivity. Moreover, our development program was ready to move from the examination of "model" RNA molecules to targeted RNA therapeutic use sequences (Costales et al., 2020).

Challenges of Targeting Small Molecules of Drugs

The Bacterial Ribosome and Developing New Antibiotics

> Ribonucleoprotein complexes are appealing pharmacological targets because they containwell-defined binding sites made up of both RNA and proteins. But new anti-bacteria areurgently needed to overcome the problem of drug resistance, which severely limits theeffectiveness of the current antibiotic arsenal (S. Levin, 1993).

The atomic features of an antibiotic class, aminoglycosides, and interaction with ribosomal RNA were originally uncovered by a pioneering NMR study. Last year, the structures of both bacterial ribosome subunits were determined using X-ray crystallography (Clemons et al., 2001). Antibiotics that bind to the big subunit do not yet have structural evidence. Antibiotics that target the 30S subunit impair one of these activities. Tetracycline, for instance, attaches to the aminoacyl tRNA site (called the A-site) and ejects. Antibiotic paromomycin helps in the translocation of the IRNA and associated mRNA during polypeptide chain elongation. The 30S is important in "decoding" the mRNA sequence by matching it to its appropriate aminoacylated tRNA (Clemons et al., 2001).

Mechanisms of RNA Recognition by Small Molecules

The ability of RNA to build complex three-dimensional structures is connected to its varied biological activities. RNA's purine and pyrimidine bases are functionally rich molecular

structures that may be read directly via particular hydrogen bonds and stacking interactions. It is, however, less chemically varied, because proteins are made of 20 distinct amino acids. The structure and chemical diversity of RNA motifs, as well as whether they can be identified by small molecules with the same specificity as proteins are critical concerns for drug development. Some of these concerns have been answered by current research on ribosomal RNA but others remain unanswered. Ribosomal RNA is mostly made up of irregular double helix stems and loops that are arranged in a complicated tertiary structure. Clefts between the various helices and loops help small-molecule medicines to generate well-defined binding sites. Tetracycline, for example, is a neutral polycycle with polar functions on one side that binds two locations inside the 30S ribosomal subunit. The bulk of contacts are created between the drug's polar groups and the sugar-phosphate backbone of adjacent RNA helices at both locations (Brodersen et al., 2000). Aminoglycoside antibiotics are positively charged, flexible compounds made up of multiple amino sugar rings connected in a linear array that form polar interactions with the RNA backbone and base groups in the main groove. Pactamycin's two aromatic rings form stacking contacts with one another and with a neighboring stem-loop. In this study, two adenine bases are displaced from helix 44 by paromomycin and neomycin B. Hygromycin B does not occur in this case at any base displacement and sequence-specific sequence reading of each core base through hydrogen bonding mediates RNA recognition almost entirely. This is the first time a drug binding to RNA is commonly accomplished by induced fit, as seen in the interaction between the HIV-1 TAR RNA regulatory element and Tat-derived peptides and organic compounds (S. Wang et al., 1998).

Researchers have discovered what happens when tiny molecules use the flexibility of the RNA structure to set a proper binding pocket. The population of various states may be affected by small molecular ligands that induce completely new conformity in the RNA objective. The

flexibility of RNA provides apparent issues with structural drug design but increases the possibility of a therapeutic intervention. Allosteric regulation can be achieved by obliging an RNA to confirm as if for the binding of neomycin B to HIV-1 TAR RNA, to be unsuited for its purpose. In the case of antibiotics that bind the RNA core of the ribosome, a therapeutic effect can also be produced through the stabilization of the RNA in a single form that cannot switch between multiple conformers. Given the negative charge of RNA molecules, electrostatic interactions are crucial for binding Cationic compounds and RNA affinity and discrimination are the well-recognized RNA-binding ligands, regulated by interaction between non-specific electrostatic forces that are essential to affinity and specific interactivities. A variety of RNAs include TARs and THE RRE HIV-1 motifs, numerous ribozymes, and human mRNAs that are preferentially bound to bacterial ribosomal RNA but are also bound to a range of non-binding RNAs. So electrostatic interactions are the double-edged sword: at the cost of diminished specialties and inefficient cellular connections they increase affinity (Sucheck et al., 2000).

Chapter 7

Conclusion & Perspectives

In this review, the RNA structures are introduced as drug targets and mediators in human diseases. Later the RNA structures are used in different ways to find the RNAs that target small molecules. In RNA drug discovery there are mainly three components, they are (i) to identify the disease-related targets, (ii) to evaluate the druggability of identified targets, and (iii) discovery of drug based on rational design and high throughput screening.

Though RNAs are introduced as the mediator in human diseases yet there is insufficient knowledge about the interaction of RNA small molecules and RNA 3D structures which leads

to holding back on the progress of RNA targeted drug discovery and its durability. To elaborate, only linezolid is the single class of small molecule targeting RNA that is used clinically. Some basic principles are preferred for the assessment of RNA small molecule interactions and RNA druggability to guide the discovery of the RNA-targeted drug.

One of the components of the drug discovery is high throughput screening and rational drug design, though it was initially designed for the protein targets by making modifications in the RNA structures and specific physicochemical properties it can also be used for targeting RNA. Similarly, ligands with high affinity and specificity mostly target the RNAs which has ligand-binding pockets and stable structure. In addition, the interaction of proteins and RNAs can also provide a rigid structure and functional significance. To understand the mechanism of the diseases related to RNA structures the study on the development of the effectiveness of the RNA therapeutics which successfully targets the RNA structure can lead to a new path. The ongoing study on RNA structure with the novel technologies helps to enlighten us about the regulatory part of the RNA structure in human diseases. Lastly, many new essential RNA therapeutic drugs and targets can be obtained by pioneering techniques based on new structures of RNA.

The above review shows the usage of RNA drugs and targeting the RNA small molecules and therapeutics. In my point of view, a broad change can be made to create the modified RNA-targeted drugs. There could be more attention given to high throughput screening and rational drug discovery to ensure the ability of the ligands to bind with the specific targets. Moreover, RNA drugs can easily be obtained than proteins when it is known about composing a high-quality RNA target. For the reason that the principles of the RNA structures and folding are easy in comparison with the proteins, also the RNA motifs that are to be targeted are far-reaching.

References

Aagaard, L., & Rossi, J. J. (2007). RNAi therapeutics: Principles, prospects and challenges. *Advanced Drug Delivery Reviews*, *59*(2–3), 75–86. https://doi.org/10.1016/j.addr.2007.03.005

Aartsma-Rus, A. (2017). FDA Approval of Nusinersen for Spinal Muscular Atrophy Makes 2016 the Year of Splice Modulating Oligonucleotides. *Nucleic Acid Therapeutics*, *27*(2), 67–69. https://doi.org/10.1089/nat.2017.0665

Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics*, *5*(4), 505–515. https://doi.org/10.1021/mp800051m

Amado, R. G., Mitsuyasu, R. T., Rosenblatt, J. D., Ngok, F. K., Bakker, A., Cole, S., Chorn, N., Lin, L. S., Bristol, G., Boyd, M. P., Macpherson, J. L., Fanning, G. C., Todd, A. V., Ely, J. A., Zack, J. A., & Symonds, G. P. (2004). Anti-human immunodeficiency virus hematopoietic progenitor cell-delivered ribozyme in a phase I study: Myeloid and lymphoid reconstitution in human immunodeficiency virus type-1-infected patients. *Human Gene Therapy*, *15*(3), 251–262. https://doi.org/10.1089/104303404322886101

Ambros, V. (2004). The functions of animal microRNAs. *Nature*, *431*(7006), 350–355. https://doi.org/10.1038/nature02871

Bader, A. G., Brown, D., & Winkler, M. (2010). The promise of microRNA replacement therapy. *Cancer Research*, *70*(18), 7027–7030. https://doi.org/10.1158/0008-5472.CAN-10-2010

Bartel, D. P. (2009). MicroRNAs: Target Recognition and Regulatory Functions. *Cell*, *136*(2), 215–233. https://doi.org/10.1016/j.cell.2009.01.002

Bennett, C. F. (2019). Therapeutic antisense oligonucleotides are coming of age. *Annual*

Review of Medicine, *70*, 307–321. https://doi.org/10.1146/annurev-med-041217-010829

Bernat, V., & Disney, M. D. (2015). RNA Structures as Mediators of Neurological Diseases and as Drug Targets. *Neuron*, *87*(1), 28–46. https://doi.org/10.1016/j.neuron.2015.06.012

Bernstein, E., Kim, S. Y., Carmell, M. A., Murchison, E. P., Alcorn, H., Li, M. Z., Mills, A. A., Elledge, S. J., Anderson, K. V., & Hannon, G. J. (2003). Dicer is essential for mouse development. *Nature Genetics*, *35*(3), 215–217. https://doi.org/10.1038/ng1253

Bertrand, J. R., Pottier, M., Vekris, A., Opolon, P., Maksimenko, A., & Malvy, C. (2002). Comparison of antisense oligonucleotides and siRNAs in cell culture and in vivo. *Biochemical and Biophysical Research Communications*, *296*(4), 1000–1004. https://doi.org/10.1016/S0006-291X(02)02013-2

Birmingham, A., Anderson, E. M., Reynolds, A., Ilsley-Tyree, D., Leake, D., Fedorov, Y., Baskerville, S., Maksimova, E., Robinson, K., Karpilow, J., Marshall, W. S., & Khvorova, A. (2006). 3’ UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nature Methods*, *3*(3), 199–204. https://doi.org/10.1038/nmeth854

Blount, K. F., & Breaker, R. R. (2006). Riboswitches as antibacterial drug targets. *Nature Biotechnology*, *24*(12), 1558–1564. https://doi.org/10.1038/nbt1268

Bosc, N., Meyer, C., & Bonnet, P. (2017). The use of novel selectivity metrics in kinase research. *BMC Bioinformatics*, *18*(1), 1–12. https://doi.org/10.1186/s12859-016-1413-y

Bramsen, J. B., & Kjems, J. (2012). Development of therapeutic-grade small interfering rnas by chemical engineering. *Frontiers in Genetics*, *3*(AUG), 1–22. https://doi.org/10.3389/fgene.2012.00154

Brodersen, D. E., Clemons, W. M., Carter, A. P., Morgan-Warren, R. J., Wimberly, B. T., & Ramakrishnan, V. (2000). The structural basis for the action of the antibiotics

tetracycline, pactamycin, and hygromycin B, on the 30S ribosomal subunit. *Cell*, *103*(7), 1143–1154. https://doi.org/10.1016/S0092-8674(00)00216-6

Castanotto, D., & Rossi, J. J. (2009). The promises and pitfalls of RNA-interference-based therapeutics. *Nature*, *457*(7228), 426–433. https://doi.org/10.1038/nature07758

Chi, X., Gatti, P., & Papoian, T. (2017). Safety of antisense oligonucleotide and siRNA-based therapeutics. *Drug Discovery Today*, *22*(5), 823–833. https://doi.org/10.1016/j.drudis.2017.01.013

Chu, E., & Allegra, C. J. (1993). Regulation of thymidylate synthase in human colon cancer cells treated with 5-fluorouracil and interferon-gamma. *Advances in Experimental Medicine and Biology*, *339*, 143–151. https://doi.org/10.1007/978-1-4615-2488-5_16

Clemons, W. M., Brodersen, D. E., McCutcheon, J. P., May, J. L. C., Carter, A. P., Morgan-Warren, R. J., Wimberly, B. T., & Ramakrishnan, V. (2001). Crystal structure of the 30 S ribosomal subunit from Thermus thermophilus: Purification, crystallization and structure determination. *Journal of Molecular Biology*, *310*(4), 827–843. https://doi.org/10.1006/jmbi.2001.4778

Corey, D. R. (2007). Chemical modification: The key to clinical application of RNA interference? *Journal of Clinical Investigation*, *117*(12), 3615–3622. https://doi.org/10.1172/JCI33483

Costales, M. G., Childs-Disney, J. L., Haniff, H. S., & Disney, M. D. (2020). How We Think about Targeting RNA with Small Molecules. *Journal of Medicinal Chemistry*, *63*(17), 8880–8900. https://doi.org/10.1021/acs.jmedchem.9b01927

Costales, M. G., Haga, C. L., Velagapudi, S. P., Childs-Disney, J. L., Phinney, D. G., & Disney, M. D. (2017). Small Molecule Inhibition of microRNA-210 Reprograms an Oncogenic Hypoxic Circuit. *Journal of the American Chemical Society*, *139*(9), 3446–

3455. https://doi.org/10.1021/jacs.6b11273

Crooke, S. T., Witztum, J. L., Bennett, C. F., & Baker, B. F. (2018). RNA-Targeted Therapeutics. *Cell Metabolism*, *27*(4), 714–739. https://doi.org/10.1016/j.cmet.2018.03.004

Damase, T. R., Sukhovershin, R., Boada, C., Taraballi, F., Pettigrew, R. I., & Cooke, J. P. (2021). The Limitless Future of RNA Therapeutics. *Frontiers in Bioengineering and Biotechnology*, *9*(March), 1–24. https://doi.org/10.3389/fbioe.2021.628137

Datlinger, P., Rendeiro, A. F., Schmidl, C., Krausgruber, T., Traxler, P., Klughammer, J., Schuster, L. C., Kuchler, A., Alpar, D., & Bock, C. (2017). Pooled CRISPR screening with single-cell transcriptome readout. *Nature Methods*, *14*(3), 297–301. https://doi.org/10.1038/nmeth.4177

Demeshkina, N., Jenner, L., Westhof, E., Yusupov, M., & Yusupova, G. (2012). A new understanding of the decoding principle on the ribosome. *Nature*, *484*(7393), 256–259. https://doi.org/10.1038/nature10913

DeNap, J. C. B., Thomas, J. R., Musk, D. J., & Hergenrother, P. J. (2004). Combating drug-resistant bacteria: Small molecule mimics of plasmid incompatibility as antiplasmid compounds. *Journal of the American Chemical Society*, *126*(47), 15402–15404. https://doi.org/10.1021/ja044207u

Ding, H., Schwarz, D. S., Keene, A., Affar, E. B., Fenton, L., Xia, X., Shi, Y., Zamore, P. D., & Xu, Z. (2003). Selective silencing by RNAi of a dominant allele that causes amyotrophic lateral sclerosis. *Aging Cell*, *2*(4), 209–217. https://doi.org/10.1046/j.1474-9728.2003.00054.x

Disney, M. D., Labuda, L. P., Paul, D. J., Poplawski, S. G., Pushechnikov, A., Tran, T., Velagapudi, S. P., Wu, M., & Childs-Disney, J. L. (2008). Two-dimensional

combinatorial screening identifies specific aminoglycoside-RNA internal loop partners. *Journal of the American Chemical Society*, *130*(33), 11185–11194. https://doi.org/10.1021/ja803234t

Doench, J. G., & Sharp, P. A. (2004). Specificity of microRNA target selection in translational repression. *Genes and Development*, *18*(5), 504–511. https://doi.org/10.1101/gad.1184404

Donlic, A., & Hargrove, A. E. (2018). Targeting RNA in mammalian systems with small molecules. *Wiley Interdisciplinary Reviews: RNA*, *9*(4), 1–21. https://doi.org/10.1002/wrna.1477

Dowdy, S. F. (2017). Overcoming cellular barriers for RNA therapeutics. *Nature Biotechnology*, *35*(3), 222–229. https://doi.org/10.1038/nbt.3802

Drewry, D. H., Wells, C. I., Andrews, D. M., Angell, R., Al-Ali, H., Axtman, A. D., Capuzzi, S. J., Elkins, J. M., Ettmayer, P., Frederiksen, M., Gileadi, O., Gray, N., Hooper, A., Knapp, S., Laufer, S., Luecking, U., Michaelides, M., Müller, S., Muratov, E., … Willson, T. M. (2017). Progress towards a public chemogenomic set for protein kinases and a call for contributions. *PLoS ONE*, *12*(8), 1–20. https://doi.org/10.1371/journal.pone.0181585

Duan, Z., & Yu, A. M. (2016). Bioengineered non-coding RNA agent (BERA) in action. *Bioengineered*, *7*(6), 411–417. https://doi.org/10.1080/21655979.2016.1207011

Dulla, K., Aguila, M., Lane, A., Jovanovic, K., Parfitt, D. A., Schulkens, I., Chan, H. L., Schmidt, I., Beumer, W., Vorthoren, L., Collin, R. W. J., Garanto, A., Duijkers, L., Brugulat-Panes, A., Semo, M., Vugler, A. A., Biasutto, P., Adamson, P., & Cheetham, M. E. (2018). Splice-Modulating Oligonucleotide QR-110 Restores CEP290 mRNA and Function in Human c.2991+1655A>G LCA10 Models. *Molecular Therapy - Nucleic*

Acids, *12*(September), 730–740. https://doi.org/10.1016/j.omtn.2018.07.010

Elbashir, S. M., Lendeckel, W., & Tuschl, T. (2001). RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes and Development*, *15*(2), 188–200. https://doi.org/10.1101/gad.862301

Farh, K. K. H., Grimson, A., Jan, C., Lewis, B. P., Johnston, W. K., Lim, L. P., Burge, C. B., & Bartel, D. P. (2005). Biochemistry: The widespread impact of mammalian microRNAs on mRNA repression and evolution. *Science*, *310*(5755), 1817–1821. https://doi.org/10.1126/science.1121158

Fedorov, Y., Anderson, E. M., Birmingham, A., Reynolds, A., Karpilow, J., Robinson, K., Leake, D., Marshall, W. S., & Khvorova, A. (2006). Off-target effects by siRNA can induce toxic phenotype. *Rna*, *12*(7), 1188–1196. https://doi.org/10.1261/rna.28106

Filipowicz, W. (2000). Imprinted expression of small nucleolar RNAs in brain: Time for RNomics. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(26), 14035–14037. https://doi.org/10.1073/pnas.97.26.14035

Fomenko, A. I., Donchenko, G. V., & Stepanenko, S. P. (1996). Effect of withdrawal of phenazepam and nicotinamide on the state of the systems of reception of benzodiazepines and NAD. *Ukrain'skyi Biokhimichnyi Zhurnal*, *68*(5), 24–25.

Fotin-Mleczek, M., Duchardt, K. M., Lorenz, C., Pfeiffer, R., Ojkić-Zrna, S., Probst, J., & Kallen, K. J. (2011). Messenger RNA-based vaccines with dual activity induce balanced TLR-7 dependent adaptive immune responses and provide antitumor activity. *Journal of Immunotherapy*, *34*(1), 1–15. https://doi.org/10.1097/CJI.0b013e3181f7dbe8

García, C. A. (2018). En el 2017, cada día se accidentaron 1.800 personas en su trabajo. *El Tiempo*, 72–83. http://www.eltiempo.com/economia/sectores/panorama-de-los-accidentes-de-trabajo-en-colombia-en-2017-189464

Georgiadis, C., Preece, R., Nickolay, L., Etuk, A., Petrova, A., Ladon, D., Danyi, A., Humphryes-Kirilov, N., Ajetunmobi, A., Kim, D., Kim, J. S., & Qasim, W. (2018). Long Terminal Repeat CRISPR-CAR-Coupled "Universal" T Cells Mediate Potent Anti-leukemic Effects. *Molecular Therapy*, *26*(5), 1215–1227. https://doi.org/10.1016/j.ymthe.2018.02.025

Grimm, D., Streetz, K. L., Jopling, C. L., Storm, T. A., Pandey, K., Davis, C. R., Marion, P., Salazar, F., & Kay, M. A. (2006). Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature*, *441*(7092), 537–541. https://doi.org/10.1038/nature04791

Grogan, J. A., Leen, S. B., & McHugh, P. E. (2012). Comparing coronary stent material performance on a common geometric platform through simulated bench testing. *Journal of the Mechanical Behavior of Biomedical Materials*, *12*, 129–138. https://doi.org/10.1016/j.jmbbm.2012.02.013

Guo, P., Coban, O., Snead, N. M., Trebley, J., Hoeprich, S., Guo, S., & Shu, Y. (2010). Engineering rna for targeted sirna delivery and medical application. *Advanced Drug Delivery Reviews*, *62*(6), 650–666. https://doi.org/10.1016/j.addr.2010.03.008

Gurumurthy, C. B., Sato, M., Nakamura, A., Inui, M., Kawano, N., Islam, M. A., Ogiwara, S., Takabayashi, S., Matsuyama, M., Nakagawa, S., Miura, H., & Ohtsuka, M. (2019). Creation of CRISPR-based germline-genome-engineered mice without ex vivo handling of zygotes by i-GONAD. In *Nature Protocols* (Vol. 14, Issue 8). Springer US. https://doi.org/10.1038/s41596-019-0187-x

Haga, Christopher L., Velagapudi, S.P., Strivelli, J.R., Yang, W.-Y., Disney, M.D., Phinney, D. G. (2015). Small Molecule Inhibition of miR-544 Biogenesis Disrupts Adaptive Responses to Hypoxia by Modulating ATM-mTOR Signaling Department of Molecular

Therapeutics , The Scripps Research Institute , Scripps Florida , Jupiter , FL 33458 , United States of America. *ACS Chemical Biology*, 1–31.

Hammond, S. M. (2006). MicroRNA therapeutics: A new niche for antisense nucleic acids. *Trends in Molecular Medicine*, *12*(3), 99–101. https://doi.org/10.1016/j.molmed.2006.01.004

Hermann, T. (2003). Chemical and functional diversity of small molecule ligands for RNA. *Biopolymers*, *70*(1), 4–18. https://doi.org/10.1002/bip.10410

Hickerson, R. P., Vlassov, A. V., Wang, Q., Leake, D., Ilves, H., Gonzalez-Gonzalez, E., Contag, C. H., Johnston, B. H., & Kaspar, R. L. (2008). Stability study of unmodified siRNA and relevance to clinical use. *Oligonucleotides*, *18*(4), 345–354. https://doi.org/10.1089/oli.2008.0149

Hornung, V., Guenthner-Biller, M., Bourquin, C., Ablasser, A., Schlee, M., Uematsu, S., Noronha, A., Manoharan, M., Akira, S., De Fougerolles, A., Endres, S., & Hartmann, G. (2005). Sequence-specific potent induction of IFN-α by short interfering RNA in plasmacytoid dendritic cells through TLR7. *Nature Medicine*, *11*(3), 263–270. https://doi.org/10.1038/nm1191

Houseley, J., & Tollervey, D. (2009). The Many Pathways of RNA Degradation. *Cell*, *136*(4), 763–776. https://doi.org/10.1016/j.cell.2009.01.019

Huang, X., Ding, L., Bennewith, K. L., Tong, R. T., Welford, S. M., Ang, K. K., Story, M., Le, Q. T., & Giaccia, A. J. (2009). Hypoxia-Inducible mir-210 Regulates Normoxic Gene Expression Involved in Tumor Initiation. *Molecular Cell*, *35*(6), 856–867. https://doi.org/10.1016/j.molcel.2009.09.006

Hutvagner, G., & Simard, M. J. (2008). Argonaute proteins: Key players in RNA silencing. *Nature Reviews Molecular Cell Biology*, *9*(1), 22–32. https://doi.org/10.1038/nrm2321

Jackson, A. L., Burchard, J., Leake, D., Reynolds, A., Schelter, J., Guo, J., Johnson, J. M., Lim, L., Karpilow, J., Nichols, K., Marshall, W., Khvorova, A., & Linsley, P. S. (2006). Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. *Rna*, *12*(7), 1197–1205. https://doi.org/10.1261/rna.30706

Jackson, A. L., Burchard, J., Schelter, J., Chau, B. N., Cleary, M., Lim, L., & Linsley, P. S. (2006). Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. *Rna*, *12*(7), 1179–1187. https://doi.org/10.1261/rna.25706

Jarvest, R. L., Hibbs, M. J., Jaworski, D. D., O'Hanlon, P. J., Pope, A. J., Rittenhouse, S., Sheppard, R. J., Slater-Radosti, C., Worby, A., Berge, J. M., Berry, V., Boyd, H. F., Brown, M. J., Elder, J. S., Forrest, A. K., Fosberry, A. P., & Gentry, D. R. (2002). Nanomolar inhibitors of Staphylococcus aureus methionyl tRNA synthetase with potent antibacterial activity against gram-positive pathogens. *Journal of Medicinal Chemistry*, *45*(10), 1959–1962. https://doi.org/10.1021/jm025502x

John, B., Enright, A. J., Aravin, A., Tuschl, T., Sander, C., & Marks, D. S. (2004). Human microRNA targets. *PLoS Biology*, *2*(11). https://doi.org/10.1371/journal.pbio.0020363

Johnson, S. M., Grosshans, H., Shingara, J., Byrom, M., Jarvis, R., Cheng, A., Labourier, E., Reinert, K. L., Brown, D., & Slack, F. J. (2005). RAS is regulated by the let-7 microRNA family. *Cell*, *120*(5), 635–647. https://doi.org/10.1016/j.cell.2005.01.014

Kelly, T. J., Souza, A. L., Clish, C. B., & Puigserver, P. (2011). A Hypoxia-Induced Positive Feedback Loop Promotes Hypoxia-Inducible Factor 1α Stability through miR-210 Suppression of Glycerol-3-Phosphate Dehydrogenase 1-Like. *Molecular and Cellular Biology*, *31*(13), 2696–2706. https://doi.org/10.1128/mcb.01242-10

Khorkova, O., & Wahlestedt, C. (2017). Oligonucleotide therapies for disorders of the nervous system. *Nature Biotechnology*, *35*(3), 249–263.

https://doi.org/10.1038/nbt.3784

Khvalevsky, E. Z., Gabai, R., Rachmut, I. H., Horwitz, E., Brunschwig, Z., Orbach, A., Shemi, A., Golan, T., Domb, A. J., Yavin, E., Giladi, H., Rivkin, L., Simerzin, A., Eliakim, R., Khalaileh, A., Hubert, A., Lahav, M., Kopelman, Y., Goldin, E., … Galun, E. (2013). Mutant KRAS is a druggable target for pancreatic cancer. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(51), 20723–20728. https://doi.org/10.1073/pnas.1314307110

Khvorova, A., Reynolds, A., & Jayasena, S. D. (2003). Functional siRNAs and miRNAs exhibit strand bias. *Cell*, *115*(2), 209–216. https://doi.org/10.1016/S0092-8674(03)00801-8

Kirk, S. R., & Tor, Y. (1999). tRNA(Phe) binds aminoglycoside antibiotics. *Bioorganic and Medicinal Chemistry*, *7*(9), 1979–1991. https://doi.org/10.1016/S0968-0896(99)00170-4

Kole, R., Krainer, A. R., & Altman, S. (2012). RNA therapeutics: Beyond RNA interference and antisense oligonucleotides. *Nature Reviews Drug Discovery*, *11*(2), 125–140. https://doi.org/10.1038/nrd3625

Leader, B., Baca, Q. J., & Golan, D. E. (2008). Protein therapeutics: A summary and pharmacological classification. *Nature Reviews Drug Discovery*, *7*(1), 21–39. https://doi.org/10.1038/nrd2399

Lee, S., Phuan, P. W., Felix, C. M., Tan, J. A., Levin, M. H., & Verkman, A. S. (2017). Nanomolar-Potency Aminophenyl-1,3,5-triazine Activators of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Chloride Channel for Prosecretory Therapy of Dry Eye Diseases. *Journal of Medicinal Chemistry*, *60*(3), 1210–1218. https://doi.org/10.1021/acs.jmedchem.6b01792

Lennox, K. A., & Behlke, M. A. (2016). Cellular localization of long non-coding RNAs

affects silencing by RNAi more than by antisense oligonucleotides. *Nucleic Acids Research*, *44*(2), 863–877. https://doi.org/10.1093/nar/gkv1206

Levin, A. A. (2019). Treating Disease at the RNA Level with Oligonucleotides. *New England Journal of Medicine*, *380*(1), 57–70. https://doi.org/10.1056/nejmra1705346

Levin, S. (1993). The crisis in antibiotic resistance. *Infectious Diseases in Clinical Practice*, *2*(1), 53. https://doi.org/10.1097/00019048-199301000-00013

Liu, J., Carmell, M. A., Rivas, F. V., Marsden, C. G., Thomson, J. M., Song, J. J., Hammond, S. M., Joshua-Tor, L., & Hannon, G. J. (2004). Argonaute2 is the catalytic engine of mammalian RNAi. *Science*, *305*(5689), 1437–1441. https://doi.org/10.1126/science.1102513

Lundin, K. E., Højland, T., Hansen, B. R., Persson, R., Bramsen, J. B., Kjems, J., Koch, T., Wengel, J., & Smith, C. I. E. (2013). Biological Activity and Biotechnological Aspects of Locked Nucleic Acids. In *Advances in Genetics* (Vol. 82). https://doi.org/10.1016/B978-0-12-407676-1.00002-0

Lundstrom, K. (2018). Latest development on RNA-based drugs and vaccines. *Future Science OA*, *4*(5). https://doi.org/10.4155/fsoa-2017-0151

Mandal, M., Boese, B., Barrick, J. E., Winkler, W. C., & Breaker, R. R. (2003). Riboswitches control fundamental biochemical pathways in Bacillus subtilis and other bacteria. *Cell*, *113*(5), 577–586. https://doi.org/10.1016/S0092-8674(03)00391-X

Marques, J. T., & Williams, B. R. G. (2005). Activation of the mammalian immune system by siRNAs. *Nature Biotechnology*, *23*(11), 1399–1405. https://doi.org/10.1038/nbt1161

Martinez, J., Patkaniowska, A., Urlaub, H., Lührmann, R., & Tuschl, T. (2002). Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell*, *110*(5), 563–574. https://doi.org/10.1016/S0092-8674(02)00908-X

Mayer, G., & Famulok, M. (2006). High-throughput-compatible assay for glmS riboswitch metabolite dependence. *ChemBioChem*, *7*(4), 602–604. https://doi.org/10.1002/cbic.200500490

McClorey, G., Moulton, H. M., Iversen, P. L., Fletcher, S., & Wilton, S. D. (2006). Antisense oligonucleotide-induced exon skipping restores dystrophin expression in vitro in a canine model of DMD. *Gene Therapy*, *13*(19), 1373–1381. https://doi.org/10.1038/sj.gt.3302800

Mello, C. C. (2007). *Return to the RNAi World : Rethinking Gene Expression and Evolution (Nobel Lecture)***. *01605*, 6985–6994. https://doi.org/10.1002/anie.200701713

Miller, V. M., Gouvion, C. M., Davidson, B. L., & Paulson, H. L. (2004). Targeting Alzheimer's disease genes with RNA interference: An efficient strategy for silencing mutant alleles. *Nucleic Acids Research*, *32*(2), 661–668. https://doi.org/10.1093/nar/gkh208

Morrissey, D. V., Lockridge, J. A., Shaw, L., Blanchard, K., Jensen, K., Breen, W., Hartsough, K., Machemer, L., Radka, S., Jadhav, V., Vaish, N., Zinnen, S., Vargeese, C., Bowman, K., Shaffer, C. S., Jeffs, L. B., Judge, A., MacLachlan, I., & Polisky, B. (2005). Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. *Nature Biotechnology*, *23*(8), 1002–1007. https://doi.org/10.1038/nbt1122

Novina, C. D., Murray, M. F., Dykxhoorn, D. M., Beresford, P. J., Riess, J., Lee, S. K., Collman, R. G., Lieberman, J., Shankar, P., & Sharp, P. A. (2002). siRNA-directed inhibition of HIV-1 infection. *Nature Medicine*, *8*(7), 681–686. https://doi.org/10.1038/nm725

O'Rourke, J. R., & Swanson, M. S. (2009). Mechanisms of RNA-mediated disease. *Journal of Biological Chemistry*, *284*(12), 7419–7423. https://doi.org/10.1074/jbc.R800025200

Ogle, J. M., Brodersen, D. E., Clemons, J., Tarry, M. J., Carter, A. P., & Ramakrishnan, V. (2001). Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science*, *292*(5518), 897–902. https://doi.org/10.1126/science.1060612

Papachristofilou, A., Hipp, M. M., Klinkhardt, U., Früh, M., Sebastian, M., Weiss, C., Pless, M., Cathomas, R., Hilbe, W., Pall, G., Wehler, T., Alt, J., Bischoff, H., Geißler, M., Griesinger, F., Kallen, K. J., Fotin-Mleczek, M., Schröder, A., Scheel, B., … Zippelius, A. (2019). Phase Ib evaluation of a self-adjuvanted protamine formulated mRNA-based active cancer immunotherapy, BI1361849 (CV9202), combined with local radiation treatment in patients with stage IV non-small cell lung cancer. *Journal for ImmunoTherapy of Cancer*, *7*(1), 1–14. https://doi.org/10.1186/s40425-019-0520-5

Pereira, P., Pedro, A. Q., Tomás, J., Maia, C. J., Queiroz, J. A., Figueiras, A., & Sousa, F. (2016). Advances in time course extracellular production of human pre-miR-29b from Rhodovulum sulfidophilum. *Applied Microbiology and Biotechnology*, *100*(8), 3723–3734. https://doi.org/10.1007/s00253-016-7350-x

Praszkier, J., & Pittard, A. J. (2002). Pseudoknot-dependent translational coupling in repBA genes of the IncB plasmid pMU720 involves reinitiation. *Journal of Bacteriology*, *184*(20), 5772–5780. https://doi.org/10.1128/JB.184.20.5772-5780.2002

Praszkier, Judy, & Pittard, A. J. (2005). Control of replication in I-complex plasmids. *Plasmid*, *53*(2), 97–112. https://doi.org/10.1016/j.plasmid.2004.12.005

Ray, P. S., Jia, J., Yao, P., Majumder, M., Hatzoglou, M., & Fox, P. L. (2009). A stress-responsive RNA switch regulates VEGFA expression. *Nature*, *457*(7231), 915–919. https://doi.org/10.1038/nature07598

Robb, G. B., Brown, K. M., Khurana, J., & Rana, T. M. (2005). Specific and potent RNAi in the nucleus of human cells. *Nature Structural and Molecular Biology*, *12*(2), 133–137.

https://doi.org/10.1038/nsmb886

Roccaro, A. M., Sacco, A., Purschke, W. G., Moschetta, M., Buchner, K., Maasch, C., Zboralski, D., Zöllner, S., Vonhoff, S., Mishima, Y., Maiso, P., Reagan, M. R., Lonardi, S., Ungari, M., Facchetti, F., Eulberg, D., Kruschinski, A., Vater, A., Rossi, G., … Ghobrial, I. M. (2014). SDF-1 inhibition targets the bone marrow niche for cancer therapy. *Cell Reports*, *9*(1), 118–128. https://doi.org/10.1016/j.celrep.2014.08.042

Sahel, D. K., Mittal, A., & Chitkara, D. (2019). CRISPR/Cas system for genome editing: Progress and prospects as a therapeutic tool. *Journal of Pharmacology and Experimental Therapeutics*, *370*(3), 725–735. https://doi.org/10.1124/jpet.119.257287

Sahin, U., Karikó, K., & Türeci, Ö. (2014). MRNA-based therapeutics-developing a new class of drugs. *Nature Reviews Drug Discovery*, *13*(10), 759–780. https://doi.org/10.1038/nrd4278

Sandbrink, J. B., & Shattock, R. J. (2020). RNA Vaccines: A Suitable Platform for Tackling Emerging Pandemics? *Frontiers in Immunology*, *11*(December), 1–9. https://doi.org/10.3389/fimmu.2020.608460

Schlesinger, R. W. (1977). Viral Replication in Cell Cultures. *Dengue Viruses*, *617*(December), 49–72. https://doi.org/10.1007/978-3-7091-8466-0_7

Sucheck, S. J., Greenberg, W. A., Tolbert, T. J., & Wong, C. H. (2000). Design of small molecules that recognize RNA: Development of aminoglycosides as potential antitumor agents that target oncogenic RNA sequences. *Angewandte Chemie - International Edition*, *39*(6), 1080–1084. https://doi.org/10.1002/(SICI)1521-3773(20000317)39:6<1080::AID-ANIE1080>3.0.CO;2-B

Sztuba-Solinska, J., Chavez-Calvillo, G., & Cline, S. E. (2019). Unveiling the druggable RNA targets and small molecule therapeutics. *Bioorganic and Medicinal Chemistry*,

27(10), 2149–2165. https://doi.org/10.1016/j.bmc.2019.03.057

Takeshita, F., & Ochiya, T. (2006). Therapeutic potential of RNA interference against cancer. *Cancer Science*, *97*(8), 689–696. https://doi.org/10.1111/j.1349-7006.2006.00234.x

ter Brake, O., 't Hooft, K., Liu, Y. P., Centlivre, M., von Eije, K. J., & Berkhout, B. (2008). Lentiviral vector design for multiple shRNA expression and durable HIV-1 inhibition. *Molecular Therapy*, *16*(3), 557–564. https://doi.org/10.1038/sj.mt.6300382

Thomas, J. R., Liu, X., & Hergenrother, P. J. (2005). Size-specific ligands for RNA hairpin loops. *Journal of the American Chemical Society*, *127*(36), 12434–12435. https://doi.org/10.1021/ja051685b

Vater, A., & Klussmann, S. (2015). Turning mirror-image oligonucleotides into drugs: The evolution of Spiegelmer® therapeutics. *Drug Discovery Today*, *20*(1), 147–155. https://doi.org/10.1016/j.drudis.2014.09.004

Wagner, M. J., Mitra, R., Mcarthur, M. J., Baze, W., Barnhart, K., Wu, S. Y., Rodriguez-Aguayo, C., Zhang, X., Coleman, R. L., Lopez-Berestein, G., & Sood, A. K. (2017). Preclinical mammalian safety studies of EPHARNA (DOPC Nanoliposomal EphA2-Targeted siRNA). *Molecular Cancer Therapeutics*, *16*(6), 1114–1123. https://doi.org/10.1158/1535-7163.MCT-16-0541

Walter, F., Pütz, J., Giegé, R., & Westhof, E. (2002). Binding of tobramycin leads to conformational changes in yeast tRNAAsp and inhibition of aminoacylation. *EMBO Journal*, *21*(4), 760–768. https://doi.org/10.1093/emboj/21.4.760

Wang, S., Huber, P. W., Cui, M., Czarnik, A. W., & Mei, H. Y. (1998). Binding of neomycin to the TAR element of HIV-1 RNA induces dissociation of Tat protein by an allosteric mechanism. *Biochemistry*, *37*(16), 5549–5557. https://doi.org/10.1021/bi972808a

Wang, Z., Rao, D. D., Senzer, N., & Nemunaitis, J. (2011). RNA interference and cancer

therapy. *Pharmaceutical Research*, *28*(12), 2983–2995. https://doi.org/10.1007/s11095-011-0604-5

Watts, J. K., & Corey, D. R. (2012). Silencing disease genes in the laboratory and the clinic. *Journal of Pathology*, *226*(2), 365–379. https://doi.org/10.1002/path.2993

Winkler, W. C., Nahvi, A., Roth, A., Collins, J. A., & Breaker, R. R. (2004). Control of gene expression by a natural metabolite-responsive ribozyme. *Nature*, *428*(6980), 281–286. https://doi.org/10.1038/nature02362

Wood, H. (2018). Traumatic brain injury induces transmissible tau pathology. *Nature Reviews Neurology*, *14*(10), 570–571. https://doi.org/10.1038/s41582-018-0062-3

Yi, R., Doehle, B. P., Qin, Y., Macara, I. G., & Cullen, B. R. (2005). Overexpression of Exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs. *Rna*, *11*(2), 220–226. https://doi.org/10.1261/rna.7233305

Yu, A. M., Choi, Y. H., & Tu, M. J. (2020). RNA drugs and RNA targets for small molecules: Principles, progress, and challenges. *Pharmacological Reviews*, *72*(4), 862–898. https://doi.org/10.1124/pr.120.019554

Yu, A. M., Jian, C., Yu, A. H., & Tu, M. J. (2019). RNA therapy: Are we using the right molecules? *Pharmacology and Therapeutics*, *196*, 91–104. https://doi.org/10.1016/j.pharmthera.2018.11.011

Zamore, P. D., Tuschl, T., Sharp, P. A., & Bartel, D. P. (2000). RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell*, *101*(1), 25–33. https://doi.org/10.1016/s0092-8674(00)80620-0

Zhang, B., & Mallapragada, S. (2011). The mechanism of selective transfection mediated by pentablock copolymers; Part II: Nuclear entry and endosomal escape. *Acta Biomaterialia*, *7*(4), 1580–1587. https://doi.org/10.1016/j.actbio.2010.11.033

Zhou, J., & Rossi, J. (2017). Aptamers as targeted therapeutics: Current potential and challenges. *Nature Reviews Drug Discovery*, *16*(3), 181–202. https://doi.org/10.1038/nrd.2016.199

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