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Non-Coding RNA-Based Therapy

ABSTRACT

In recent years, some of the physiological and pathophysiological roles of non-coding RNAs (ncRNAs) have been discovered. These discoveries have been pivotal in providing insight on the basis of various diseases where ncRNAs are aberrantly expressed. Moreover, they are being studied in translational research to use these mechanisms for 'non-coding RNA based therapy'. The main players being investigated in non-coding RNA-based therapies include antisense oligonucleotides, locked nucleic acid anti-miRs, miRNA sponges, miRNA mimics, siRNAs and ribozymes.

INTRODUCTION

Only approximately 1.5% of the human genome encodes protein sequence,[1] the rest was once labelled as 'dark matter'. Now we know that a major part of this once proverbial genomic 'dark matter' is represented by non-coding RNAs (ncRNAs), which increase in number with increasing complexity of an organism. Based on their size, ncRNAs are roughly grouped into two major classes, namely, small ncRNA and long ncRNA (lncRNA). MicroRNAs (miRNAs, mi-R, approximately 22 nucleotides long) and transcription initiation RNAs (tiRNAs, 18 nucleotides long) are two examples of the first class. In contrast, lncRNAs, which resemble mRNA transcripts, range from 200 nucleotides to approximately 100 kilobases.[2]

Of the small ncRNAs, microRNAs are the most studied. Indeed, more than 2,000 microRNAs have been described. Their sequence is evolutionary conserved between species, and can be intergenic, intronic or polycistronic. MicroRNA target messenger RNA (mRNA) and inhibit their expression, specifically by either causing repression of their translation or bring about their degradation. So the function of a microRNA is to post-transcriptionally regulate gene expression by the above two mechanisms. Their binding to the targeted mRNA is partially complementary, specifically and usually by a 7 nucleotide sequence called the 'seed sequence'. However, usually there might be more complementary nucleotides but this is not often necessary as long as the seed sequence binds. One microRNA can have more than 100 target genes.

In contrast lncRNAs show poor evolutionary conservation between species but this does not mean that the function is not, because if they fold in the same manner and if the 3-dimensional configuration of the RNA is the same, they can still possibly bind to the same proteins or perform the same function. More than 30,000 lncRNAs have been described. Their expression can be highly tissue specific. Many lncRNAs are localized in the nucleus, but not always. Like miRNA, lncRNA can be anywhere in the genome. They have been described to be intergenic, intronic, bidirectional, sense and anti-sense.

LncRNAs control various cellular functions. For example one function is acting like an 'RNA decoy', binding to transcription factors (TF) (that usually would bind to double-stranded DNA) and thus preventing the TF from binding to the DNA. Other lncRNAs act as 'microRNA sponges', binding to microRNAs and thus prevent the latter from binding to their target mRNA. They can also act as a 'scaffold' to bring proteins together ensuring their interaction. Interestingly, some lncRNAs interact directly with DNA and then recruit chromatin modifiers which then result in genes being turned on or off. In the cytoplasm, lncRNAs can bind to mRNA and so prevent their translation or splicing, or bring about mRNA degradation. The name 'non-coding' is misleading as it implicates that these non-coding RNAs do not code proteins. Van Heesch et al.[3] have discovered that some lncRNAs, which have already been described to have well-defined noncoding functions, also code for functional microproteins. And to make things more complex, Khan et al.[4] found that protein coding genes code for a type of non-coding RNA, called circular RNA by a process called 'backsplicing'.

Research on these noncoding RNAs is showing that they play pivotal roles in cellular homeostasis, cellular growth and development, cellular differentiation, metabolism, apoptosis, aging, and immune regulation. Their exploration has revealed that they are important for health when they function physiologically but cause disease when aberrantly expressed or dysregulated. This has further spurred translational research of these bio-molecules for their clinical utility as disease biomarkers and molecular targets or 'bullets' in medical therapies. This essay deals with the latter, specifically therapy, aimed at or based on, non-coding RNA.

Some of the main players being investigated in non-coding RNA-based therapies include antisense oligonucleotides (ASOs), locked nucleic acid anti-miRs, micro-RNA sponges, micro-RNA mimics, small interfering RNAs (siRNAs) and ribozymes.

ANTISENSE OLIGONUCLEOTIDES (ASOs)

ASOs are short, synthetic, single-stranded oligodeoxynucleotides that interfere with RNA targets e.g. binding to mRNA preventing its translation into protein.

FDA has approved inostersen (Tegsedi®) and golodirsen (Vyondys 53™)as ASO-based therapeutics. Inotersen is indicated for the treatment of hereditary transthyretin amyloidosis (hATTR).[5] It binds to transthyretin (TTR) mRNA, and so inhibits the synthesis of TTR protein in the liver, thus reducing amyloid deposition. Vyondys 53™ is indicated for patients with Duchenne Muscular Dystrophy (DMD) with a mutation of the DMD gene that can have its exon 53 skipped.[6] Specifically, golodirsen binds to exon 53 of the dystrophin pre-mRNA which causes exclusion of exon 53 during mRNA processing. This exon 53 skipping produces a truncated dystrophin protein which still localizes to the sacrolemma in muscle fibres.

Research with ASOs in the field of cardiology is also ongoing. Patients with chronic ischaemic heart failure have increased levels of the cardiac microRNA-132-3p (miR-132). Clinical trial NCT04045405 tested CDR132L, a miR-132 inhibitor, specifically an antisense oligonucleotide. The results show that the miR-132 ASO decreases and reverses heart failure and is safe.[7]

LOCKED NUCLEIC ACID (LNA) ANTI-MIR

A 'locked nucleic acid anti-miR' is a synthetic oligonucleotide that targets specific miRs to cause antisense-based gene silencing.

In a study on mice, Boon et al.[8] used a LNA antisense oligonucleotide to silence miR-29. They showed that increased expression of miR-29 in aortas of old mice is associated with underexpression of many extracellular matrix (ECM) proteins (e.g. elastin, collagens, fibrillins), leading to formation of aortic aneurysms. By using the LNA antisense oligonucleotide they silenced the miR29, allowing ECM proteins to be expressed and thus avoiding aortic aneurysm formation.

MRG-110 is a synthetic LNA antisense oligonucleotide that targets and inhibits miR-92a. miR-92a is an anti-angiogenic miRNA and thus when inhibited increases angiogenesis in multiple organ systems. Corrie et al.[9] showed that MRG-110 speeds up healing of acute and chronic wounds of the skin in mice and pigs. The authors also proposed MRG-110 for a phase one clinical trial, which recruited 42 participants, and was completed in 2019 (ClinicalTrials.gov Identifier: NCT03603431). The trial showed that MRG-110 is safe and well tolerated, supporting the conduct of additional clinical studies.

Dysregulation of several miRNAs has been shown to be involved in the pathogenesis and progression of many malignancies. For example, in colocrectal adenocarcinoma, miR-21 is elevated,[10] and in hepatocellular carcinoma (HCC), miR-23b is high.[11] Studies using LAN anti-miR on malignant cell lines of these cancers suppressed the high levels of the culprit miR and controlled cell proliferation and cell invasion. Such studies have prompted the start of clinical trials.

Indeed, in a phase one clinical trial (ClinicalTrials.gov Identifier: NCT00466583),[12] a LNA antisense oligonucleotide against the hypoxia-inducible factor-1α was tested in patients with solid tumours or lymphoma refractory to treatment. However, the trial was terminated when the sponsor stopped the development of this agent.

LNA anti-miRs are also being studied for their potential use in treatment of parasitic infections where the parasite, like Leishmania[13] and Toxoplasma gondii,[14] uses complex strategies to evade the immune response. It was found that in these infections a specific microRNA is over-expressed, and when these detrimental microRNas are targeted by LNA anti-miR, apoptosis of the host macrophages occurs killing the parasite inside.

Lindholm et al.[15] used LNA anti-sense oligonucleotide to target and degrade the messenger RNA (mRNA) that codes the 'proprotein convertase subtilisin/kexin type 9' (PCSK9) protein in monkeys. PCSK9 causes the degradation of the LDL receptor and thus increases LDL-C level. Given that there were no toxicological side-effects, the authors proposed that LNA antisense oligonucleotides against PCSK9 can be a potential treatment for elevated high LDL-C.

This led to a first-in-human randomized, placebo-controlled trial (ClinicalTrials.gov Identifier: NCT01350960) of a LNA antisense inhibitor of PCSK9 (which in the trial was labelled SPC5001). But the trial was terminated because SPC5001 caused injection site reactions and renal toxicological side-effects.[16] The authors thus concluded that further research was needed to figure exactly the mechanisms involved in the development of the adverse reactions.

miR-155 is mechanistically involved in the formation of blood and lymphoid cells. miR-155 is elevated in specific lymphomas and leukemias, and is believed to promote the growth and survival of the cancerous cells. Clinical trials with identifiers NCT02580552 and NCT03713320 tested cobomarsen (MRG-106), a LAN that targets miR-155 with the aim to treat these blood diseases. The clinical trials showed safety and efficacy even though clinical trial NCT03713320 was terminated early because of financial reasons. In 2020, the FDA granted an 'orphan drug designation' to cobomarsen for patients suffering with T-cell lymphoma.

miRNA SPONGES

miRNA sponges are noncoding RNA molecules that bind miRNAs and thus prevent the latter from binding to their target mRNAs. Together with miRNAs, these endogenous miRNA sponges (also known as competing endogenous RNAs, ceRNAs) are involved in the regulation of gene expression and hence in cellular protein output.[17] They are called sponges as an analogy because they 'soak up' miRNAs. Two major types of endogenous sponges are circular RNAs and long non-coding RNAs.

As a proof-of-principle study for the potential clinical use of circular RNAs as microRNA sponges, Jost et al.[18] engineered circRNA sponges in vitro to soak up microRNA-122 in hepatocyte cell cultures infected with Hepatitis C Virus (HCV), which is a single-stranded RNA virus. The virus uses miRNA-122 by binding its RNA to it and this protects its

degradation and so helps to complete its life-cycle in the infected liver cells. By sponging these protective miRNA-122, the engineered circRNA inhibited the viral replication.

The research on circRNA-based therapeutics is still in its infancy but it is promising.[19] The latter is based on the fact that thousands of circRNAs have been reported to be expressed, that are highly cell-type specific, and they are resistant to exonucleolytic decay.

MICRO-RNA MIMICS

MicroRNA mimics are synthetic miRNAs that are used to restore endogenous miRNAs that are down-regulated. Thus, they have clinical therapeutic potential in various diseases like inflammation, cardiovascular diseases, neurological disorders, cancer and fibrotic conditions, where miRNAs are down-regulated.[20]

There are various studies on miR-29, which is under-expressed in fibrotic conditions like systemic sclerosis,[21] idiopathic pulmonary fibrosis,[22] and scar formation. Montogomery et al.[23] showed that a miR-29 mimic protected and even reversed pulmonary fibrosis in a mouse model of the latter condition. In a phase two clinical trial (ClinicalTrials.gov Identifier: NCT03601052),remlarsen (MRG-201), a miR-29 mimic, was studied for its efficacy and safety profile in human volunteers with a tendency towards keloids. The trial was conducted to investigate whether the miR-29 mimic, given intradermally, could reduce fibrous scar tissue formation. The results demonstrated that Remlarsen was effective.[24]

Another showcase of the potential of mi-R mimics is the clinical trial investigating TargomiRs (ClinicalTrials.gov Identifier: NCT02369198). TargomiRs are targeted mini-cells (nonliving bacterial minicells) containing a mi-R mimic. Specifically an miR-16 mimic was used. miR-16 is under-expressed in various cancers, and when its level is restored it has tumour suppressive activity. In the clinical trial the targets were malignant pleural mesothelioma and non-small lung cancer that over-expressed EGFR (epidermal growth factor receptor). The TargomiRs were injected intravenously and they found their way to the cancer cells because they had an anti-EGFR bispecific antibody that docked to the EGFR-expressing cancer cells and then 'unloaded' their miR-16 mimic cargo. This method is being refined further.[25]

siRNAs

A small interfering RNA (siRNA) is a double-stranded small RNA that targets a specific mRNA for degradation. In the past they have been used to knockdown genes in mammalian cells to understand gene function but nowadays siRNAs are also being utilized in siRNA therapeutics. Indeed, two siRNA, patisiran and givosiran, have been approved by FDA for medical use.

In 2018, FDA approved patisiran to treat adults with polyneuropathy of transthyretin-mediated amyloidosis, a rare, autosomal dominant disease. The approval was based on the significant improvements of the patients in the Phase III APOLLO clinical trial (ClinicalTrials.gov Identifier: NCT03997383).

In 2019, the FDA approved givosiran for adults with acute hepatic porphyria (AHP) after its evaluation in the clinical trial ENVISION (ClinicalTrials.gov Identifier: NCT03338816). Givosiran targets and causes the degradation of the mRNA that codes for aminolevulinate synthase 1 (ALAS1) in hepatocytes. This results in reduced levels of circulating aminolevulinic acid (ALA) and porphobilinogen (PBG), that are the neurotoxic intermediates which are responsible for the disease.

siRNAs are also showing promising results in Age-related Macular Degeneration (AMD) and glaucoma.[26] Bevasiranib is a siRNA that targets the mRNA for VEGF (Vascular Endothelial Growth Factor), the protein that promotes the growth of blood vessels. Thus, the aim here is to slow the growth and leakage from the abnormal blood vessels in the wet type of macular degeneration.

RIBOZYMES

A ribozyme is an RNA molecule that is catalytically active. The definition can be broadened to include RNA-protein complexes where the RNA fraction is the only part that is catalytic.

Zhong et al.[27] demonstrated how such ribozymes can be used in gene therapy, making the latter more safe and efficient. They engineered a type of ribozyme to act as an RNA on-switch of the therapeutic transgene.

In the phase two clinical trial (ClinicalTrials.gov Identifier: NCT00074997) an anti-HIV ribozyme (OZ1) was transduced in autologous CD34+ hematopoietic progenitor cells which were then re-administered to participants with chronic Human Immunodeficiency Virus Type 1 (HIV-1) infection. The OZ1 group had a higher CD4+ lymphocyte counts than the placebo arm and the procedure was shown to be safe.[28]

CONCLUSION

Non-coding RNA-based therapy is still in its infancy but is showing great therapeutic promise. Indeed, several RNA-targeted therapeutic projects are in the pipeline and various biotech and pharmaceutical companies are involved. - The two main challenges that must be finely addressed to bring these projects from the bench to the bedside, are stability and delivery. Most RNA molecules are relatively unstable and this is being addressed by their chemical modification. The correct delivery of the RNA to the target organ/s is also being established through the use of nanoparticles, liposome-like particles, or by joining the RNA or the particle to a specific molecule that will bind to a specific receptor on the targeted cells.

Presently many proteins cannot be targeted using small molecule based therapy. Nonetheless, non-coding RNA-based therapy has the potential to create a new class of drugs which will then modulate a multitude of targets. Thus, over the next several years, with further development and progress, noncoding RNA-based therapeutics will definitely be a transformative strategy in therapy as we know it.

References can be accessed on **www.thesynapse.net**