# Micro RNA Mimics And Antagonists

Nabajit Das, Naveen Tripathi, Sukant Khurana

**Abstract:** Gene regulation is vital for life and it involves plethora of mechanisms, including microRNA (miRNA) based RNA inhibition. Messenger RNA (mRNA) inhibition by miRNA requires less sequence specificity than inhibition by small inhibitory RNA (siRNA) and has different set of enzymes required for processing. The regulation and richness of RNA inhibition and specifically miRNA action is just beginning to be studied, while much work has undergone in the study of synthesis and processing of miRNA. More than 1800 unique human miRNAs have been computationally predicted and several have been experimentally validated. Given their ability to act as sequence specific regulators of the transcriptome, miRNAs have potential in therapeutics and diagnostics. We specifically focus on synthesis of current therapeutic applications of miRNA. We discuss the two different strategies in miRNA treatment: mimics and antagonists, and bring forward the promises and perils of miRNA therapy in its journey from lab to medicine.

**Index Terms**: antagonists, clinical trial, diseases, drugs, therapeutics, gene, mimics, miRNA.

## 1 INTRODUCTION

GENE regulation is necessary to sustain the fine-tuned orchestra of life and give unique identity and function to different cells. Our bodies have many different cell types but these cells have the same genome as they all came from the same zygote. What makes cells different from each other is how genes are regulated. Understanding differential gene regulation can shed light on both the identities and functions of cells, their differential sensitivities to different signals and sturdiness of function in the face of vastly different expressions[1-3].Different regulatory and epigenetic mechanisms partly enable different identities and functions of cells. Micro Ribonucleic Acids (miRNAs) are small RNA molecules that regulate genes, but are too tiny to code for proteins. They work like Small interfering RNA (siRNA) but do not require as high a degree of sequence complementarity; and different sets of enzymes are involved in their processing than siRNA[4].The first reported miRNA, *lin-4* was first discovered in *Caenorhabditis elegans* in 1993 by Victor Ambros' group and was found to be regulating timely developmental events[5]. This discovery was followed by several thousand microRNAs in almost all metazoan studied for miRNA[6-8]. With implications for the treatment of cancer, diabetes and brain disorders, miRNAs can play an important role in the development of future therapeutics and diagnostics.

## 2 REVIEW

### 2.1 Status and unsolved questions on the genesis of MicroRNAs

MicroRNA synthesis and processing has been described in great detail in several other works[9-11], so here we would only briefly touch upon the topic to provide the necessary background for the understanding of mimics and antagonists.


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MicroRNAs, the smallest of all small eukaryotic RNAs, suppress messages of mRNA by RNA silencing. Genesis of miRNAs begins in the nucleus, where RNA polymerase II (Pol II) transcribes primary transcripts including ones containing miRNA sequences. Such primary miRNA transcripts (pri-miRNA) get processed by an enzyme Drosha, which removes the tails of the primary miRNA, leaving a shorter stem loop structure known as pre-miRNA. Pre-miRNA associates with an exportin complex (RAN GTP) and enters the cytoplasm [12]. Once it reaches the cytoplasm, pre-miRNA is released from exportin complex and associates with DICER for further processing[13-16]. DICER cleaves the loop structure resulting in an asymmetrical double stranded RNA(miRNA) of 19-23 nucleotides[15, 17]. This double stranded RNA associates with RNA induced silencing complex (RISC complex)[15]. MiRNAs get unbound inside the RISC complex and then guide the RISC to conserved recognition sites of target mRNA[10]. Binding of RISC to the target mRNA triggers the silencing. Between different metazoans there is variability in the genesis of microRNAs[18]. MicroRNAs are physically clustered and often exist within protein coding and long non coding RNA genes[19, 20]. For example, roughly 1/3 of microRNAs lie in the introns of protein coding gene where the host gene drives the expression of the microRNAs[21, 22]. Given the fact that RISC complex holds on to the driver strand once a target mRNA is degraded, the answer which is yet not clear is: what is the fate of the RISC complex after the first round of target mRNA degradation? One possibility is that the complex gets ready for the next possible target mRNA match. If so, then how many times is the cycle repeated inside the cytoplasm? It would be interesting to find out the destiny of the passenger strand, which leaves the RISC complex before the miRNA held inside the RISC finds its target. There are reports that the enzymes in the cytoplasm degrade the passenger strand[23] but there is a small, as yet unexplored possibility that the passenger strand might also find a complementarity in the mRNA strand in the cytoplasm and block any other mRNA processing. We also do not know if the single stranded free passenger miRNA strand can again get bound to the free ends of career strands in RISC complex, diminishing its function.

### 2.2 Status of development in miRNA therapeutic approaches

Inappropriate expression of miRNA leads to numerous diseases in humans[24, 25], such as miR-195 contributing to postnatal loss of cardiac regenerative capacity [26]; miR-1,

miR-133a/b and miR-208 being dysregulated in human myocardial infraction[27]; and miR-33 negatively regulating cholesterol homeostasis[28]. These molecules need to be systematically blocked in order to regain normal functionality in the diseased condition. Many of them are in pre-clinical testing and might end up being drugs in the near future. Depending on the expression pattern in pathological condition, there are two major kinds of disease therapies: miRNA mimics[29, 30]and miRNA antagonists[31, 32]. Figure 1 summarizes the effect of miRNA mimics and antagonists on the target gene protein expression. To better understand their biological roles, we can use tools to increase or decrease miRNA availability and function. MiRNA mimics are used to restore loss of function of miRNAs in the diseased tissues. Such mimics carry out processes in the same way as the mature miRNAs in any cell. They are double stranded miRNA-like non-natural RNA fragments designed to silence genes. Mimics results in an increase in the number of RISC complexes in the cytoplasm. Coupled with the RISC complex, the mimic acts on the target mRNA to block its post-transcriptional activity. In this regard, miRNA can be used to replace traditional gene therapy approaches. For example, miR-34 is known to inhibit human pancreatic cancer tumor-initiating cells[33], down-regulation of which may lead to a pathological conditions. A mimic of miR-34 is the first of its kind to enter a phase I clinical trial after having been shown to globally downregulate target mRNA. No side effect has been reported so far [34]. Another example that gives a clearview of the beneficiary role of miRNAs in human disease and why we need mimics to restore their loss, is that loss of *let-7* function enhances lung tumour formation[35]. Mimicking the nature of this candidate miRNA regains the appropriate functioning. *Let-7*mimic is being tested in non-human primates[35, 36]. Successful development of a miRNA mimic requires the RNA molecule to be capable of entering the RISC complex and targeting the genes of interest. Oligonucleotide engineering for miRNA mimics is conducted in a manner that would retain sequence complementarity with target mRNAs. The original function to act as a guide sequence within RISC is retained by such mimics to cope with the loss of endogenous miRNA in pathological conditions[37]. This is known as miRNA replacement therapy[38].It helps regain identical miRNA activities that are reduced or missing in the diseased state. In addition to mimics, we posit that the transcribing DNA sequence can also be inserted in the genome or introduced as plasmids to induce a gain in the function of miRNAs in various organisms. In humans, this approach would likely not find much ground in the immediate future due to ethical concerns but can be of use in economically important organisms. In a diametrically opposite approach, use of miRNA antagonists (anti-miR or antagomiR or antisense oligonucleotides) is an effective strategy to block endogenous miRNAs that acquire a gain in function in human disease. MicroRNA antagonists are short, single stranded oligonucleotide molecules that target the active miRNA in question with partial or full complementarity, before the endogenous miRNA reaches its target mRNA. This triggers a break-down of the endogenous miRNA by forming a duplex structure of the endogenous and exogenous miRNA. For example, miRNA-122 is believed to promote the Hepatitis C Virus (HCV) [39]. Systematic silencing of miR-122 with anti-miR-122 locked nucleic acids (LNA) in non-human primates with the Hepatitis C infection triggers a breakdown of endogenous miR-122, the causative agent in the diseased phenotype[40, 41]. This candidate is ahead of all other anti-miRNAs in the drug development pipeline currently being tested in phase II of a clinical trial[42]. In order to determine the right miRNA antagonist construct, a thorough *in vitro* and *in vivo* examination is needed. Such an examination, with various modifications, can reveal the function of individual microRNAs. MiRNA antagonists are single-stranded antisense oligonucleotides that are completely or partially complementary to the target miRNA[43, 44]. The result of any such exogenous oligonucleotide injection is endogenous miRNA degradation [45]. The fate of degraded mRNA needs to be systematically studied. Compared to siRNA, where 100% sequence complementarity is required for functioning, miRNAs provide far more sturdy tools as even in much lower complementarity; they are capable of RNA inhibition. This feature of lower complementarity would also drive the price of therapies down, and their effectiveness higher because one does not have to design a personalized RNA inhibition approach using miRNAs. A potential flip side of the coin might be the additional testing required for potentially “off-target” effects. Of the eight novel drug candidates listed in table1, Miravirsen, a miR-122 blocker, is leading the table, after having successfully passed phase I of a clinical trial and is currently being tested in phase II. Miravirsen is a product of SantarisPharma. Listed second in the table, MRX 34 is designed to mimic the functions of the natural miR-34 and is the first of its kind to enter Phase I of a clinical trial. MRX 34 is a product of Mirna Therapeutics Inc. TargomiRs, a mimic of miR-116 is in the clinical phase 1 which has a role in malingnant pleural mesothelioma and non-small cell lung cancer. Another potential drug candidate listedin the table1, miR-Rxlet-7 is designed to mimic functions of natural let-7. It is next in line to enter a Phase I clinical trial and is currently being tested in non-human primates. The other advanced potential anti-miRNA drug candidates miR-195, miR-208, miR-33, and miR-155 are yet to enter clinical trials. We gathered information on clinical trials from https://clinicaltrials.gov/.

### 2.3 Figures and Tables


***Fig.1.*** *Effect of microRNA, microRNA mimics, and antagonists on protein expression: Hypothetical expression levels of same protein for control, mimics and antagonists to show the effect of miRNAs.*

## 4 Conclusion

Both approaches of mimics and antagonists have started showing promising results. We expect several more miRNA based therapies to enter clinical trials in the coming years and to make it to market soon. While progress in miRNA therapeutics has been noticeable, for continued progress in the field, unanswered questions relating to the biology of miRNA will need to be answered. We present a few outstanding unsolved issues here. Identification of the trigger

factors that promote miRNA expression across species has not begun in earnest yet. The regulatory network of miRNA, feedback based on miRNA levels and activity remain unexplored. Epigenetic regulation of inhibitory RNAs including miRNA remains a mystery at present. Evolution of miRNA and overall inhibitory RNA machinery also remain largely unexplored. Detailed, structured function studies of Drosha and Dicer are much needed. Even with a smaller sequence than siRNA incomplementarity match, miRNA can regulate target mRNAs. This raises the possibility of regulation of more than one mRNA. This can be double edged sword, enabling design of miRNA that works for larger population segments of whole human race on one hand and a concern for potential "off-target" on the other hand. Systematic analysis of both "on-target" effects and "off-target" side-effects, if such side-effects are indeed the case for miRNA therapy, would be required for case by case basis. Additionally, routes of administration, especially enteral and parenteral, remain to be studied systematically for the degradation and half life of these potential therapeutic agents. At present, the long-term and short-term effects of molecular medicine *in-vivo* are still not entirely clear; as the question of how many gene transcripts can be regulated by one miRNA is an open one. In summary, we expect several promises of miRNA therapeutics to start bearing fruit and expect basic biology research on miRNA to be filled with several surprises.

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***TABLE 1***

*MICRORNAS (MIMICS AND ANTAGONISTS) WITH THERAPEUTIC PROMISE*

| Drug Program | Key Targets | Pathological Role | Clinical Trial Status |
|---|---|---|---|
| MRX 34 (mimic of miR-34) | BCl2, E2F3, HDAC1, MET, MEK1, CDK 4/6, PDGFR-α, WNT 1/3, NOTCH-1 | Liver cancer | Phase I |
| miR-Rxlet-7 (mimic of let-7) | RAS, MYC, HMGA2, TGFBR1, MYCN, Cyclin D2, IL6, ITGB3 | Lung cancer | Pre-clinical* |
| TargomiRs (mimic of mir-16) | Bcl-2 and CCND1 | Malignant Pleural Mesothelioma and non-small cell lung cancer | Phase I |
| Miravirsen (antagonist of miR-122) | miR-122 | Liver infection (Hepatitis C Virus) | Phase II |
| Unassigned/ Undisclosed(antagonist of miR-195) | miR-195 | Heart Regeneration | Pre-clinical* |
| Unassigned/Undisclosed (antagonist of miR-208) | miR-208 | Chronic heart failure | Pre-clinical* |
| Unassigned/Undisclosed (antagonist of miR-33) | miR-33 | Regulates cholesterol metabolism | Pre-clinical* |
| Unassigned/Undisclosed (antagonist of miR-155) | TF-c/ebp β | Chronic Inflammatory disease | Pre-clinical* |

*Pre-clinical testing in non-human primates.

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