

# The Therapeutic Potential of microRNAs

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A novel mechanism of action, the ability to function as master regulators of the genome and an apparent lack of adverse events in normal tissue make microRNA a promising technology for current and future therapeutic development.

It was only 10 years ago that the first human microRNA (miRNA) was discovered, and yet a miRNA-based therapeutic has already entered Phase 2 clinical trials. This rapid progress from discovery to development reflects the importance of miRNAs as critical regulators in human disease, and holds the promise of yielding a new class of therapeutics that could represent an attractive addition to the current drug pipeline of Big Pharma.

## miRNAs AS DRUG TARGETS

MicroRNAs (miRNAs) are 15-22 nucleotide, short, non-coding RNAs that have emerged as critical regulators of gene expression – affecting a multitude of biological processes including cell proliferation, differentiation, survival and motility. They are conserved from plants to man and are encoded by their respective genes. miRNA genes are defined in separate gene loci, or alternatively can be found within introns and exons of other genes. A stepwise maturation process involving transcription, nuclear export and progressive cleavage leads to an approximately 19-nucleotide double-stranded RNA molecule that enters a large cytoplasmic protein

complex to regulate gene expression at the post-transcriptional level (see Figure 1). In this way, the protein complex – also referred to as RNA-induced silencing complex (RISC) – facilitates the interaction of the mature miRNA with mRNAs that contain complementary sequences. As a result, mRNAs targeted by the miRNA are either degraded or remain ‘silenced’ so that they are no longer translated into proteins. On average, a given miRNA can regulate several hundred transcripts whose effector molecules function at various sites within cellular pathways and networks. Because of this, miRNAs are able to switch instantly between cellular

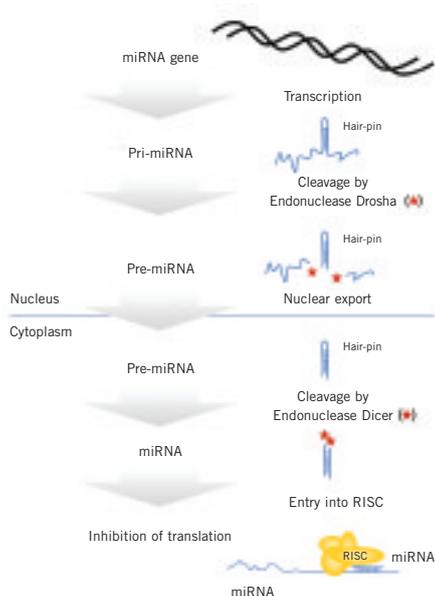
programmes and are therefore often viewed as master regulators of the human genome.

As with other regulatory molecules, miRNAs are frequently subject to change during the development of human disease. To date, there is hardly any disease that has not revealed significant differences in miRNA expression compared with normal tissue. These include cancer, obesity, diabetes, inflammation, neurological disorders such as Alzheimer’s and Parkinson’s disease, as well as cardiovascular and autoimmune diseases. The extensive involvement of miRNAs across human disease illustrates that many molecular key players have been overlooked for many years, but are now available for the study and development of new therapeutic strategies. The principles that apply to developing miRNA-based therapies remain the same as for other targeted therapies that take the path from drug target to drug. For instance, target identification and validation are key to selecting miRNAs that are causally involved in the disease process. Furthermore, diligent drug development is necessary to assure satisfactory efficacy, specificity and lack of toxicity. However, since miRNAs constitute a class of drug targets unrelated to any others, new ancillary technologies and methods are also required.

## miRNAs IN CANCER

Many of the miRNAs identified to date have been associated with cancer. A comparison of tumour tissue with normal tissue has shown that miRNA genes are frequently located at fragile sites of the human genome and subject to chromosomal rearrangement, gene amplification and deletion (1). In addition, epigenetic mechanisms can lead to improper expression of miRNA genes. As a result, many miRNAs are aberrantly expressed in tumour tissue, leading to a potentially faulty regulation of their target mRNAs. While many of these miRNAs have no impact on cancer development, select miRNAs directly contribute to the oncogenic phenotype. Often, the misregulation of these miRNAs is evident in a broad range of different cancer types, indicating that they can function as conventional tumour suppressors and

**Figure 1:** Genesis and mechanism of miRNAs





oncogenes. Examples of oncogenic miRNAs are miR-10b, miR-155, miR-21 and miR-17-92, a cluster of miRNA genes that contains 7 miRNAs. The phenomenon that cancer cells can become addicted to key oncogenes also applies to miRNAs as demonstrated for miR-21 (2). Tumour suppressor miRNAs include miR-26a, miR-126, miR-335, as well as members of the *let-7* and miR-34 families. Some of these miRNAs exert their roles at different stages of tumour development – for instance, miR-10b regulates metastasis and is found to be positively associated with high-grade malignancies. Inhibition of miR-10b prevents the metastatic dissemination of cancer cells, but does not inhibit the growth of existing metastases. Similarly, expression of miR-335 inhibits tumour cell metastasis, but fails to alter the proliferation and apoptotic rates of cancer cells. Several others are able to prevent both tumour cell proliferation and metastasis.

The strong oncogenic or tumour suppressive effects are explained by the fact that each of these miRNAs can regulate multiple cancer genes that function in separate oncogenic pathways. For instance, miR-34a represses many oncogenes that – by themselves – can induce oncogenic cellular transformation. Validated oncogenic miR-34a target genes are: c-Met, a proto-oncogene also known as hepatocyte growth factor receptor; cyclin-dependent kinase 4 (CDK4), a kinase that promotes cell-cycle progression; and BCL2 (B-cell lymphoma 2), an oncogenic protein that blocks apoptosis and is often hyperactivated in many cancer cells (see Figure 2). Of note is the fact that various pharmaceutical companies have selected each of these oncogenes as drug targets for the generation of small molecule inhibitors. However, considering that many cancer therapeutics targeting a single oncogene merely induce a modest therapeutic response, the ability to repress many oncogenes at once and across different oncogenic pathways provides a strong rationale for developing miRNA-based cancer therapeutics.

### CANCER STEM CELLS

Another attractive feature of miRNAs is the observation that they can play a critical role in cancer stem cells. Cancer stem cells are defined as a small fraction of cancer cells that have the ability to self-renew and give rise to identical daughter cells. As such, cancer stem cells are often considered to be the ‘seed’ of the tumour and have been described as more tumorigenic, metastatic and refractory to therapy. Therefore, chemoresistance and recurrence are likely due to the presence of cancer stem cells. Similar to a comparison of tumour and normal tissues, cancer stem cells carry miRNAs that are differentially expressed when compared with the bulk of tumour cells. Often, miRNAs that are already aberrantly

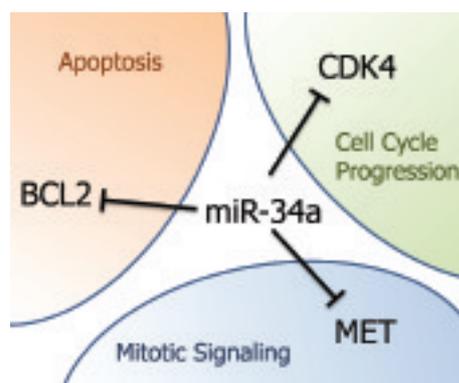
expressed in the bulk tumour show an even more pronounced difference in their expression levels in cancer stem cells. This is exemplified by *let-7* and miR-34, both of which are down-regulated in many tumour tissues and at even lower levels in cancer stem cells (3,4). Functional assays *in vitro* and *in vivo* have revealed that administration of *let-7* and miR-34 specifically interferes with the phenotypic properties of cancer stem cells. Therefore, it is tempting to speculate that therapeutic delivery of *let-7* and miR-34 will not only reduce the bulk of the tumour but also the number of viable cancer stem cells.

The involvement of miRNAs in ‘stemness’ should not come as a surprise as several miRNAs were first recognised in experiments that studied embryonic development. *let-7* was first described as a switch gene in the worm *Caenorhabditis elegans* that is temporally expressed to control developmental timing (5). It is absent in early larval stages and induced in later larval stages to signal the transition to the adult form. In contrast, expression levels of members of the oncogenic miR-17-92 cluster are high at early stages during murine lung development, but decline as development proceeds (6). Thus, there appears to be an inverse correlation between miRNAs that control stem-like tissue and those that control adult tissue – from stemness to differentiation, a process that is often reversed in cancer cells.

### THERAPEUTIC miRNA MODALITIES

In general, there are two approaches to developing miRNA-based therapeutics: miRNA antagonists and miRNA mimics. miRNA antagonists are generated to inhibit endogenous miRNAs that show a gain-of-function in diseased tissues. This approach is conceptually similar to other inhibitory therapeutics that target a single gene product such as small molecule inhibitors and short interfering RNAs (siRNAs). It usually involves the introduction of a highly chemically-modified miRNA passenger strand (anti-miR or antagomiR) that binds with high affinity to the active miRNA strand. Since binding is frequently irreversible, the new miRNA duplex is unable to be processed by RISC and/or degraded. As a potential concern, the antagonist may also non-specifically bind to other RNAs, which could result in unwanted side effects.

miRNA mimics are used to restore a loss of function. This approach, also known as ‘miRNA replacement therapy’,



**Figure 2:** Oncogenes and pathways regulated by the tumour suppressor miR-34a

Table 1: miRNAs in therapeutic development		
miRNA	Indication	Status of development
<b>miRNA antagonists</b>		
miR-122	Hepatitis C virus	Phase 2 clinical trials
miR-208/499	Chronic heart failure	Preclinical development
miR-195	Post-myocardial infarction remodelling	Preclinical development
<b>miRNA replacement</b>		
miR-34	Cancer	Preclinical development
let-7	Cancer	Preclinical development

aims to re-introduce miRNAs into diseased cells that are normally expressed in healthy cells (7). In many cases, the re-introduction of these miRNAs leads to a re-activation of pathways that are required for normal cellular welfare and block those that drive the disease. Proof-of-concept for miRNA replacement therapy has been demonstrated by mimics of tumour suppressor miRNAs that stimulate anti-oncogenic pathways, apoptosis and ultimately lead to an eradication of tumour cells (8-10). Considering that most targeted cancer therapeutics developed to date tackle a gain-of-function, much of the excitement around miRNA replacement therapy stems from the fact that it provides a new opportunity to exploit tumour suppressors. miRNA mimics can be delivered systemically using technologies that are also used for therapeutic siRNAs. Therefore, the application of miRNA mimics will face less of a delivery hurdle compared with protein-encoding plasmid DNA previously used in gene therapy. Besides, miRNA mimics are expected to be highly specific and well tolerated in normal tissues. This supposition is based on the fact that miRNA mimics carry the same sequence as the naturally occurring equivalent and are expected to target the same set of genes. Since most normal cells already express the miRNA in question, administration of miRNA mimics to normal tissue is unlikely to induce adverse events as the cellular pathways affected by the mimic are already activated or inactivated by the endogenous miRNA.

#### miRNAs IN THERAPEUTIC DEVELOPMENT

Despite the recent discovery of miRNAs, several candidates have already progressed into product and clinical development. miRNAs under investigation are those that not only yield satisfactory efficacy in various disease models, but also sufficient mechanistic data that allow a fairly accurate placement of the miRNA into disease-related pathways. According to publicly available information, the most advanced candidates are shown in Table 1. Among these are mimics of the *let-7* and miR-34 miRNAs to target a broad spectrum of solid tumours. A key target repressed by *let-7* is KRAS, an oncogene that is frequently mutated in lung cancer and other cancer types, and has escaped many previous attempts for therapeutic

intervention (11). Therapeutic delivery of *let-7* – either in the form of a *let-7* mimic or a virus – leads to a robust inhibition of tumour growth in human non-small cell lung cancer xenografts and the KRAS-G12D transgenic mouse model (12). Similarly, systemic delivery of an miR-34 mimic blocked tumour growth in mouse models of lung and prostate cancer (10,16). The therapeutic efficacy correlates with reduced proliferation and enhanced apoptotic activity of tumour cells, as well as a specific repression of CDK4, Met and BCL2 (10). In addition, therapeutic delivery of miR-34 did not induce an elevation of cytokines or liver and kidney enzymes in serum, suggesting that treatment is well tolerated and the anti-oncogenic effects are mediated by a specific mechanism of miR-34.

Other miRNAs currently in development are in the area of cardiovascular disease and include miR-208, miR-499 and miR-195. miR-208 is a miRNA that is fairly exclusively expressed in the heart. It is encoded by an intron of the alpha-myosin heavy chain ( $\alpha$ -MHC) gene and stimulates cardiomyocyte hypertrophy, fibrosis and  $\beta$ -MHC expression in response to stress and hypothyroidism. miR-208 knock-out mice were resistant to fibrosis and cardiomyocyte hypertrophy in response to induction of cardiac hypertrophy and heart failure, suggesting that an antagonistic approach to miR-208 ameliorates chronic heart disease (13). Likewise, inhibition of miR-499 – a miRNA that is functionally redundant with miR-208 in the heart and promotes slow myofibre gene programmes – favours the expression of fast-acting muscle fibres in the heart and represses  $\beta$ -MHC expression in response to stress. Another miRNA that has directly been linked to pathological cardiac growth is miR-195; it is upregulated during cardiac hypertrophy and – when over-expressed in transgenic mice – can lead to heart failure.

Although many miRNAs with therapeutic value have been discovered in genetically manifested disorders, the miRNA-based therapeutic that is most clinically advanced at the present time targets an infectious disease – hepatitis C (HCV). Specifically, a miR-122 antagonist, SPC3649, is administered to hepatocytes to block replication of the virus (14). It is generally known that viruses can hijack cellular elements to generate viral progeny; in this case, a liver-specific miRNA, miR-122, binds to two closely spaced target sites within the 5' non-coding region of the HCV genome that is necessary to maintain the abundance of viral RNA (15). Systemic administration of SPC3649 to chimpanzees chronically infected with HCV induced a long-lasting suppression of viral RNA in serum (14). Notably, viral repression remained without the emergence of escape mutants – an observation that is in stark contrast

to direct-acting antivirals. Since both miR-122 target sites are highly conserved among all HCV genotypes, the strict dependence on miR-122 might have occurred early during HCV evolution and promises to provide an effective way to treat this viral disease that is of epidemic proportions. In addition, no adverse effects associated with SPC3649 have been reported. SPC3649 is currently being evaluated in Phase 2 clinical trials and may be the first miRNA-based therapeutic that is brought to market.

## CONCLUDING REMARKS

The value of miRNAs as therapeutic targets is now widely recognised. Within 10 years, slightly more than 1,000 human unique miRNAs have been discovered and await utility in clinical applications. For many of these, extensive investigations *ex vivo* suggest therapeutic opportunities in areas of unmet medical need; some of these have already been validated in clinically-relevant animal models, and a few are in (pre)clinical development. A new mechanism of action, the ability to function as master regulators of the genome and an apparent lack of adverse events in normal tissue make 'drug target miRNA' a promising technology for current and future product development. A serious obstacle for the translation of these programmes into the clinic is the absence of an effective delivery system. The poor pharmacokinetic properties of therapeutic miRNAs define an urgent need for delivery companies to generate adjuvant carrier systems that increase stability, prevent renal clearance and enhance cellular uptake by target tissues. This task is scientifically and technically daunting, but should prove to be clinically rewarding. The recent clinical progress achieved with therapeutic siRNAs using liposomal and polymer-based delivery technologies is encouraging and is expected to jump-start clinical development of therapeutic miRNAs.

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