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REVIEW

Clinical applications of microRNAs [version 1; peer review: 2 approved]

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V1 First published: 06 Jun 2013, 2:136
<https://doi.org/10.12688/f1000research.2-136.v1>
 Second version: 23 Aug 2013, 2:136
<https://doi.org/10.12688/f1000research.2-136.v2>
 Latest published: 08 Oct 2013, 2:136
<https://doi.org/10.12688/f1000research.2-136.v3>

Abstract





MicroRNAs represent a class of small RNAs derived from polymerase II controlled transcriptional regions. The primary transcript forms one or several bulging double stranded hairpins which are processed by Drosha and Dicer into hetero-duplexes. The targeting microRNA strand of the duplex is incorporated into the RNA Induced Silencing Complex from where it silences up to hundreds of mRNA transcript by inducing mRNA degradation or blocking protein translation. Apart from involvement in a variety of biological processes, microRNAs were early recognized for their potential in disease diagnostics and therapeutics. Due to their stability, microRNAs could be used as biomarkers. Currently, there are microRNA panels helping physicians determining the origins of cancer in disseminated tumors. The development of microRNA therapeutics has proved more challenging mainly due to delivery issues. However, one drug is already in clinical trials and several more await entering clinical phases. This review summarizes what has been recognized pre-clinically and clinically on diagnostic microRNAs. In addition, it highlights individual microRNA drugs in running platforms driven by four leading microRNA-therapeutic companies.

Keywords

microRNA, siRNA, cancer, diagnosis, therapeutics, Miravirsen, Anti-miRs, MRX34

Open Peer Review

Approval Status  

	1	2
version 3 (update) 08 Oct 2013		
version 2 (update) 23 Aug 2013		 view
		
version 1 06 Jun 2013	 view	 view

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Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Hydbring P and Badalian-Very G. **Clinical applications of microRNAs [version 1; peer review: 2 approved]** F1000Research 2013, 2:136 <https://doi.org/10.12688/f1000research.2-136.v1>

First published: 06 Jun 2013, 2:136 <https://doi.org/10.12688/f1000research.2-136.v1>

MicroRNA discovery

Two decades have passed since the groundbreaking work from the laboratories of Gary Ruvkun and Victor Ambros, which demonstrated that a small temporal non-coding RNA could influence development of *Caenorhabditis elegans* by base pairing to the 3' untranslated region (3'UTR) of a coding messenger RNA (mRNA) thereby regulating its translation^{1,2}. For several years these RNA molecules were considered to be specific to *C. elegans*. A final recognition that they also played this role in additional systems, including human cells, marked the official birth of microRNAs (miRNAs)³⁻⁵.

miRNAs are separated from other small RNAs by the prediction of a hairpin fold-back structure from the precursor transcript together with expression evidence of an approximately 22 nucleotide-long mature sequence. Currently, 1600 human miRNA precursors have been deposited into [miRBase v19](#) based on analyses of RNA deep-sequencing data⁶. The nomenclature of these miRNAs is based on a “mir” or “miR” prefix with identifying numbers assigned sequentially at the time of discovery. “mir” denotes a precursor miRNA whereas “miR” denotes a mature miRNA sequence. Similar or identical sequences can be given the same number. Distinction is then accomplished by a letter (similar sequences) or numeral ending (identical sequences). For example, miR-15a and miR-15b have identical 5' ends but differ by four nucleotides in their 3' regions. In contrast, miR-16-1 and miR-16-2 are identical but encoded on two separate chromosomes, chromosome 13 and 3, respectively⁷ ([miRBase v19](#)).

Genomic location and transcription of microRNAs

miRNA genes reside either in intergenic regions, within introns of coding or non-coding genes or within exons of non-coding genes⁸. Approximately one third of miRNAs are intergenic and about one third of all miRNA loci contain clustered miRNAs ([miRBase v19](#)). Work from the laboratory of David Bartel and others demonstrated that miRNAs that are oriented in the same genomic orientation and separated by less than 50 kb but more than 0.1 kb are highly correlated in their expression patterns. These results suggest that they originated from polycistronic transcripts^{9,10}. Consistent with this, the laboratory of David Fisher showed that intronic miRNAs with independent transcriptional start sites were located substantially further away from their host gene start site, displaying a median distance of 57 kb¹¹. Predicting the number of human miRNA clusters and precursors using such cutoffs in [miRBase](#) generates 175 and 538, respectively.

The majority of miRNAs are transcribed as long primary transcripts by RNA polymerase II and many are capped and polyadenylated^{12,13}. Analysis of miRNAs residing in intergenic primary transcripts indicates that such transcripts are shorter than protein-coding transcripts with transcriptional start sites about 2 kb upstream of the pre-miRNA and polyadenylation signals 2 kb downstream¹⁴. A subset of miRNAs is transcribed by RNA polymerase III. This cluster of miRNAs is located among Alu rich regions on chromosome 19¹⁵.

While most intronic miRNAs seem to be expressed from their host-gene transcriptional machinery, promoters and transcriptional activators of intergenic miRNAs are poorly defined. In order to identify

miRNA promoters and their transcription factors, the laboratory of Richard Young set up a score system. This system overlaid genomic coordinates of tri-methylated Lysine 4 of Histone 3 (H3K4me3), their proximity to annotated miRNA sequences, expression sequence tag (EST) data and conservation between species with genome-wide association of transcription factors Oct4, Sox2, Nanog and Tcf3. The results demonstrated that miRNAs are regulated by Oct4, Sox2, Nanog and Tcf3 to the same degree as protein-coding genes¹⁶. These findings suggest that most miRNAs are transcriptionally regulated in a “protein-coding like” fashion.

Processing of microRNAs

Primary miRNA transcripts (pri-miRNAs) form hairpin bulges due to sequence complementarity. These hairpins are subject to processing by the Microprocessor complex which takes place in the nucleus²⁰. The Microprocessor consists of the RNase III enzyme Drosha and its partner DiGeorge syndrome critical region gene 8 (DGCR8). Upon binding of DGCR8 to pri-miRNA at the junction of single-stranded to double-stranded RNA, Drosha cuts the pri-miRNA hairpin 11 bp into its double-stranded stem sequence generating slightly smaller hairpins known as pre-miRNAs. Intronic miRNAs are cleaved by Drosha co-transcriptionally preceding splicing of the primary transcript¹⁷. Pre-miRNAs are exported into the cytoplasm by the exportin 5 Ran-GTP complex where they are released by GTP hydrolysis and further processed by the RNase III Dicer into an approximate 22 nucleotide duplex. Dicer counts 22 nucleotides from the 5' end of the pre-miRNA before cleaving, generating a duplex with 3' end overhangs at each side. RNA helicases facilitate unwinding of the duplex with one strand being incorporated into the targeting miRNA containing RNA Induced Silencing Complex (RISC) together with the Argonaute protein. Thermostability of the 5' ends determines that the strand will be incorporated in the complex and that the strand left out will be degraded^{8,18}.

MicroRNA functional targeting

The miRNA-RISC complex binds to 3'UTRs of mRNAs via nucleotide complementarity. Upon binding, it either induces mRNA degradation or inhibits protein translation. It is unclear to what extent mRNA degradation and translational inhibition coincide, but steady state levels suggest that mRNA degradation serves as the major final outcome for miRNA targeted transcripts¹⁹.

miRNAs bind their targets through seed- or seed-less pairing. The miRNA “seed” is located in its 5' end and expands from nucleotides 2–7. miRNAs that are interacting with their targets only through the seed region rarely produce efficient targeting. Additional base pairing of either nucleotide one, eight or both results in canonical seed targeting referred to as “7mers” or “8mers” correlating with functional outcomes. Disrupted complementarity, at any of the nucleotides between two to seven, leads to seed-less targeting. Seed-less targeting depend on compensatory complementarity along the mature miRNA sequence. In contrast, canonical seed targeting may be functional without such additional binding. During recent years, a number of prediction algorithms have been developed to track miRNA targeting based on seed pairing, overall complementarity, pairing stability, target site evolutionary conservation and UTR context²⁰.

Generally, a single miRNA is predicted to have hundreds of targets. Prediction algorithms provide significant information for selecting appropriate miRNAs for targets of interest but there are several shortcomings when one seeks the biological outcome of a specific miRNA. First, expression levels of many miRNAs vary greatly between different cell types/tissues or disease states^{10,21}, and this affects the stoichiometry of the binding of the miRNA to the target, potentially altering its impact. Secondly, the targeting capacity of a certain miRNA can be blunted by the state of its target, for example, shortened 3' UTRs in cancer cells or point-mutations in the 3' UTRs^{22,23}.

The effect of a given miRNA may not necessarily correlate with repression of its targets. 3' UTRs vary greatly in size, with many harboring binding sites for numerous endogenous miRNAs^{20,22}. Inhibiting a single miRNA is therefore likely to give non-detectable or very modest derepression of an investigated target. Finally, functional assays demonstrating mRNA or protein alterations preceded by the introduction of 'mimic miRNAs' or inhibiting endogenous miRNAs could stem from the execution of indirect biological programs and not necessarily reflect miRNA-to-target interaction.

To combat such issues, miRNA researchers have turned to biochemical methods. Pull-down assays for either the miRNA itself or the RISC-complex followed by RT-PCR, microarray or sequencing could determine if the physical interaction between a given miRNA/mRNA is present or not^{24–26}. By combining biochemical approaches with functional assays, it is possible to build confidence in specific miRNA targets. Given the huge number of predicted targets for any given miRNA, it is plausible to assume that only a fraction of predicted targets are substantially affected^{27,28}. Interestingly, a recent study overlaid interacting transcripts with the targets that were significantly downregulated due to exogenous expression of a mimic miRNA in two different cell lines. They observed that the majority of miRNA to transcript interactions may result in no or very weak functional outcomes²⁵. Such combinatory approaches are likely to be crucial in order to fully dissect miRNA functional targeting and they may ease biological network analyses by substantially reducing the number of targets.

MicroRNAs in disease diagnostics

Observations that miRNAs displayed high stability in paraffin-embedded tissues from clinical samples or in human plasma^{29,30} raised the possibility that miRNA expression analysis may be a useful tool to define disease states. An early key report from the laboratory of Todd Golub covering 217 mammalian miRNAs and several hundred samples, including clinical samples, common cancer cell lines and mouse tumors, demonstrated that miRNA profiles could distinguish a tumor's developmental origin and that miRNAs are generally downregulated in cancers (129 out of 217 miRNAs). Interestingly, poorly differentiated tumors displayed lower miRNA expression compared to tumors with a higher differentiation²¹. This report was complemented by a study looking at miRNA expression profiles in human solid tumors³¹. miRNA signatures were further used to define subtypes of cancers, such as the distinction between basal and luminal breast cancers^{32,33}. Sempere *et al.* showed that ER⁺PR⁺HER2⁺, ER⁺PR⁺HER⁺ and ER⁺PR⁺HER2⁺ breast cancer

tumors exhibited distinct miRNA patterns with miR-205 being highly expressed in triple negative breast cancers and miR-145 showing the opposite pattern. Analysis of separate cancers demonstrated miRNA profiles could predict clinical progression^{34,35}. miR-15a/16-1 act as prognostic biomarkers in chronic lymphocytic leukemia (CLL) and let-7a is a marker for lung cancers^{34,35}.

In addition to cancer, miRNA expression profiles could also be used to distinguish distinct forms of heart disease³⁶, muscular disorders³⁷ and neurodegenerative diseases³⁸. The human miRNA-associated disease database (HMDD) serves as a resource for scientists screening the constantly increasing number of miRNA profiles for a wide range of diseases³⁹. At the time of writing this review, the HMDD-database covered disease-associations from 2741 scientific publications.

Circulating microRNAs

The vast majority of miRNA expression profile reports stem from solid tissues although miRNAs can be readily detected in human plasma, serum or total blood due to their small size and high stability^{30,40}. The potential of circulating miRNAs as biomarkers in serum was indicated early by studies examining patients with diffuse large B-cell lymphoma, highlighting miR-21 as a potential biomarker⁴¹. Prostate cancer patients could be distinguished from healthy counterparts by analyzing the expression level of miR-143³⁰. These studies were followed by additional reports of miRNAs in breast cancer (using whole blood)⁴², colorectal cancer (using plasma)⁴³ and squamous cell lung cancer (using sputum)⁴⁴ patients. Interestingly, Boeri *et al.* demonstrated that circulating miRNAs may also be used for predicting purposes. They displayed miRNA signatures with strong predictive value in lung cancer patients years before the onset of disease by analyzing expression in samples taken before diagnosis, at the time of detection by computed tomography and in disease-free smokers⁴⁵.

In a massive undertaking Keller *et al.* analyzed 863 miRNAs from 454 human blood samples. The samples were from patients suffering from 14 separate diseases including lung cancer, prostate cancer, pancreatic ductal adenocarcinoma, melanoma, ovarian cancer, gastric tumors, Wilms tumor, pancreatic tumors, multiple sclerosis, chronic obstructive pulmonary disease, sarcoidosis, periodontitis, pancreatitis and myocardial infarction. On average, more than 100 miRNAs were deregulated in the blood for each disease. By utilizing this data and developing mathematical algorithms and probability plots the authors were able to accurately predict the disease in more than two thirds of individuals involved in the study⁴⁶. It should be noted that miRNA patterns in blood are unlikely to be the same between different types of blood cells. Distinct hematopoietic lineages display different miRNA-profiles⁴⁷, possibly suggesting that differences in expression in specific diseases are due to shifts in hematopoietic cell populations. This could be pronounced in diseases directly affecting the blood such as cancers spreading to the bone marrow or multiple sclerosis, an autoimmune disease associated with abnormal levels of white blood cells. Using 27 distinct cell populations with the highest variance, Keller *et al.* computed that such shifts could account for a maximum of 60% of differences in the observed miRNA profiles⁴⁶.

How are miRNAs released into the blood stream? Rechavi *et al.* demonstrated that synthetic miRNA mimics, viral miRNAs or endogenous miRNAs could be released from B-cells and taken up by T-cells upon cell contact⁴⁸. Further, Pegtel *et al.* demonstrated secretion of miRNAs from Epstein-Barr virus (EBV)-infected B-cells via exosomes, providing protection of the miRNA from RNases⁴⁹ and Yuan *et al.* reported the transfer of miRNAs from embryonic stem cells to fibroblasts via embryonic stem cell microvesicles⁵⁰. Finally, Kosaka *et al.* demonstrated that miRNA secretion occurs in a ceramide-dependent fashion, which could be blunted by knocking down nSmase2, an enzyme required for ceramide biosynthesis⁵¹.

In addition to serum/plasma, the presence of miRNAs has also been demonstrated in urine⁵² and saliva⁵³, with two miRNAs, miR-125a and miR-200a, displaying lower expression in patients with oral squamous cell carcinoma compared to healthy subjects⁵⁴.

MicroRNA profiling methods

miRNA expression profiling and disease association studies are conducted using a set of different methods including miRNA microarray platforms, quantitative real-time polymerase chain reaction (qRT-PCR), *in situ* hybridization and high throughput sequencing. Both qRT-PCR and hybridization methods are highly sensitive and quantitative. This makes them useful for analyzing small sets of miRNAs⁵⁵. It is worth noting that there are limitations with hybridization methods due to potential hybridization of the probe to pre-, pre- and mature miRNAs. Detection of mature miRNA sequences through qRT-PCR demands a stem-loop RT primer, specific primers for amplification of the cDNA and a TaqMan probe (Roche Molecular Diagnostics, Applied Biosystems)⁵⁶. *In situ* hybridization can either be represented by fluorescence *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) and detection of miRNAs as well as other non-coding RNAs is possible without protease-treatment of tissues⁵⁷.

For high-throughput studies, miRNAs are profiled using array platforms or sequencing. Next-generation sequencing technologies offer lower costs and shorter processing time making this platform highly attractive. Today, researchers frequently use sequencing techniques to profile miRNA signatures in distinct sets of tissues/diseases.

Clinical microRNA diagnostics

A major challenge in clinical diagnostics is cancers with poor differentiation. Even though these cancers account for only a few percent of malignancies, they display substantially distinct gene-signatures making it notoriously hard to trace the cell of origin despite the availability of the latest microRNA platforms⁵⁸.

Perhaps the most exciting potential of miRNAs in current diagnostics started with a study comparing miRNA expression using miRNA microarrays in 205 primary versus 131 metastatic tumors covering 22 different tumor origins. The authors developed a binary decision tree classifier based on miRNA expression with tissues displaying the highest specificity of certain miRNAs placed at the top of the tree. Following this classifier, a remarkably low number of 48 miRNAs predicted tissue origin at close to 90% accuracy when tested on a blinded set⁵⁹ (Table 1). This study, together with

subsequent studies confirming the accuracy of using miRNAs as diagnostics for tumors of unknown origin^{60,61}, was partly driven by the miRNA diagnostics company Rosetta Genomics, Israel.

Based on these reports, Rosetta Genomics is now offering a panel (miRview-mets2) to clinicians so that the origin of metastatic cancers can be identified where the primary origin of metastasis is questionable. The panel consists of a test of 64 miRNA biomarkers validated on 489 samples of which 146 represent metastatic tumors covering 42 tissues of origin⁶². In addition to the “miRview-mets2” panel, Rosetta Genomics also offers four additional clinical tests: “miRview-lung”, “miRview-squamous”, “miRview-meso” and “miRview-kidney”. MiRview-lung differentiates four types of primary lung cancer (small cell lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC and carcinoid) using eight separate miRNA biomarkers⁶³. MiRview-squamous separates non-small cell lung cancers (NSCLC) into squamous cell carcinomas and adenocarcinomas using a single miRNA, hsa-miR-205⁶⁴. MiRview-meso defines samples into a mesothelioma or non-mesothelioma origin based on three separate miRNAs⁶⁵ and miRview-kidney separates kidney cancer into its four primary types (benign oncocyoma, clear cell renal carcinoma, papillary renal carcinoma and chromophobe renal carcinoma) using six miRNAs. All clinical panels rely on the same tree-classifier as in the original paper^{59,66}.

The move of miRNA diagnostics into the clinics led by Rosetta Genomics and others (including Asuragen Austin TX, USA) may aid the use of personalized medicine in the treatment of cancers. Importantly, miRNA diagnostics are not just an additional approach for differentiated cancers but a potential breakthrough for cancers initially defined as of unknown origin.

Nucleotide polymorphism in diagnostics

In addition to more well-established approaches of analyzing miRNA expression profiles, predisposition to certain cancer types may be predicted by the existence of single nucleotide polymorphisms (SNPs). SNPs can reside in either precursor miRNAs, mature miRNAs or in 3' UTRs disrupting or creating miRNA binding sites⁶⁷.

Screening of 42 miRNA expression profiles in chronic lymphocytic leukemia (CLL) revealed the presence of mutations in 5 miRNAs; all SNPs were detected in the pri- or pre-miRNA structure. For one of these, miR-16-1, a mutation in the precursor transcript led to a significant reduction in mature miRNA expression³⁵. Other reports of SNP variants in miRNA precursors where the mutant variant correlates with an increased risk of cancer include miR-196a-2 and miR-499 in breast cancer⁶⁸, miR-196a-2 in head and neck cancer⁶⁹, whilst increased mature levels of miR-146a can lead to an earlier onset of breast and ovarian cancers⁷⁰. In addition, SNPs affecting factors in the miRNA processing machinery lead to either an increased or reduced risk of renal cell carcinoma depending on which factors were affected⁷¹.

Examples of SNPs located in 3' UTRs of miRNA target sites include the binding site of miR-221/222 and miR-146 in Kit⁷² as well as the let-7 binding site in the 3' UTR of K-Ras. The latter displays an

Table 1. Rosetta Genomics initial binary tree classifier consisting of 24 nodes giving rise to 25 leaves or defined cancer types. Each node uses 1 to 5 miRNAs for classification. Nodes giving no defined cancer types make broader distinctions. For example, Node #3 distinguishes epithelial tissues from mixed origin. Table is adapted from Rosenfeld *et al.* 2008⁵⁹.

Node	MicroRNAs used at node	Defined cancer types at node
1	miR-122a, miR-200c	Liver
2	miR-372	Testis
3	miR-200c, miR-181a, miR-205	–
4	miR-146a, miR-200a, miR-92a	–
5	miR-142-3p, miR-509	Lymph node, Melanocytes
6	miR-92b, miR-9*, miR-124a	Brain
7	miR-152, miR-130a	Meninges
8	miR-205	Thymus (type B2)
9	miR-192, miR-21, miR-210, miR-34b	–
10	miR-194, miR-382, miR-210	Lung-pleura, Kidney
11	miR-187, miR-29b	Sarcoma, Gastro Intestinal Stromal Tumors
12	miR-145, miR-194, miR-205	–
13	miR-21, let-7e	Lung (carcinoid)
14	let-7i, miR-29a	Colon
15	miR-214, miR-19b, let-7i	Stomach, Pancreas
16	miR-196a, miR-363, miR-31, miR-193a, miR-210	–
17	miR-27b, let-7i, miR-181b	Breast, Prostate
18	miR-205, miR-141, miR-193b, miR-373	–
19	miR-106b, let-7i, miR-138	Thyroid
20	miR-10b, miR-375, miR-99a	–
21	miR-205, miR-152	Lung, Bladder
22	miR-345, miR-29c, miR-182	Endometrium, Ovary
23	miR-192, miR-345	Thymus (type B3)
24	miR-182, miR-34a, miR-148b	Lung (squamous), Head and Neck

alteration associated with an increased risk of NSCLC⁷³. Further, a SNP in the miR-184 binding site in the 3' UTR of tumor necrosis factor alpha-induced protein 2 (TNFAIP2) resulted in an increased risk of squamous cell carcinoma of the head and neck⁷³.

Using available SNP data from “The 1000 Genomes Project”⁷⁴, Richardson *et al.* concluded that approximately 5% of all SNPs maps to miRNA recognition elements (MREs). Among these SNPs, 22% associated with disease phenotype⁷⁵. Interestingly, these numbers suggest that SNPs residing within MREs are likely to be under selective pressure.

MicroRNAs in therapeutics

The idea of using miRNAs in therapeutics is highly appealing after observing the outcomes from manipulating these molecules. Rather than intercepting a single target as in the case of selective protein inhibitors, miRNAs can modulate entire gene programs. Given the notion of deregulation of these molecules in a wide range of diseases

and the robust degree of accurate profiling, one would estimate the chances of mapping specific miRNAs for manipulation of a given disease with minimal side-effects to be fairly good.

miRNAs fall into the class of RNAi-based therapeutics, a concept that is not completely new, having given birth to more than 20 clinical trials so far⁷⁶, but unlike short interfering RNAs/short hairpin RNAs (siRNAs/shRNAs), which are designed to target a single transcript, modulation of miRNAs, will affect hundreds of transcripts and so would potentially be capable of shutting down entire deregulated pathways. Of course, specific targeting of disease associated transcripts is probably required since a completely random modulation of hundreds of transcripts would be too harmful for the patient. However, such issues can be relatively easily investigated from *in vitro* studies conducting genome-wide mRNA expression together with pathway enrichment analysis. To date there is only one miRNA drug in clinical trials (SPC3649: inhibitor of miR-122, Santaris Pharma, Denmark) (Figure 1). The slow progress stems

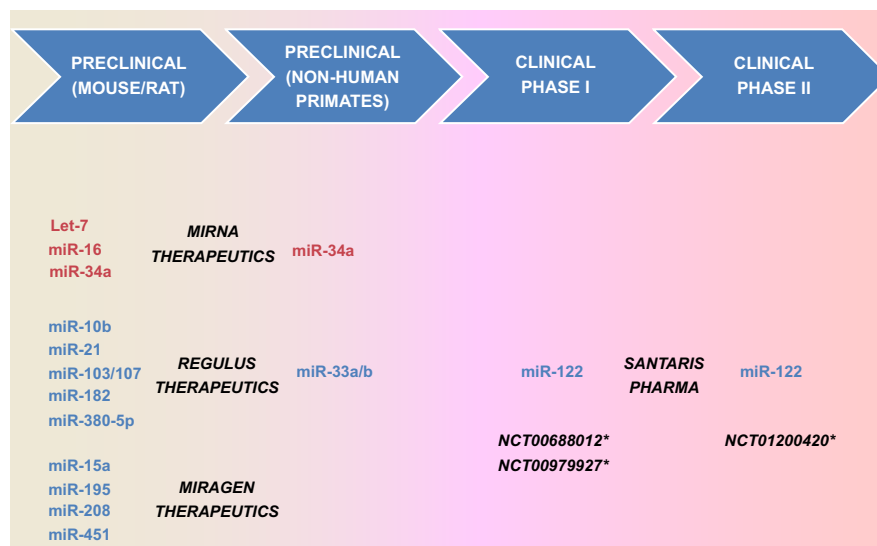


Figure 1. Leading companies developing microRNA therapeutics. The four stages at the top denote the progress of individual miRNA drugs. Red colored miRNAs indicate replacement therapy with drug mimicking the endogenous miRNA. Blue colored miRNAs indicate inhibition therapy with drug targeting the endogenous miRNA. *Santaris Pharma has completed two Phase I clinical trials and is currently in Phase II with their miR-122 targeting drug miravirsen.

from the general technical challenges with RNAi-based therapeutics including delivery, stability and avoidance of activating immune responses.

As discussed earlier in this review, endogenous miRNAs remain highly stable even when secreted into circulation. They resist nucleases by being enclosed into microvesicles or exosomes. Creating complex delivery-vehicles for miRNA therapeutics is feasible but could be time-consuming and costly. To minimize stability issues, a range of chemical modifications have been developed for siRNAs, modifications likely to be transferrable to miRNAs due to their similar structures. These include Phosphorothioate, 2'-O-methyl RNA, 2'-Fluoro-RNA and 2'-O-methoxy-ethyl RNA, all of which provide greater nuclease resistance^{76,77}. A phosphorothioate modification exchanges a nonbridging oxygen atom with a sulfur atom at the phospho-backbone of the unmodified RNA. In addition to providing nuclease resistance this also promotes RNase H-mediated cleavage of targeted transcripts. The 2'-O-methyl modification adds a methyl group (-CH₃) to the second carbon of the ribose. Likewise, 2'-Fluoro adds a fluorine atom at this position and 2'-O-methoxy-ethyl adds a methoxy-ethyl group (-C₃H₇O). All 2'-modifications increase Watson-Crick base pairing to targeted transcripts and substantiate nuclease resistance due to closer proximity between the 2' group and 3' phospho-group. In addition, a modification known as locked nucleic acid (LNA) is widely used in synthesis for miRNA inhibiting drugs, sometimes referred as antagomirs. This modification utilizes a bridge between the 2'O group and 4' carbon atom also referred to as 2'O-4'C-methylene linked ribonucleotides⁷⁷.

Even though siRNAs, and in a similar way miRNA mimics, can be stabilized through chemical modifications, their size still demands complex vehicles in order to ensure delivery to different tissues *in vivo*⁷⁸. In contrast, miRNA inhibiting drugs can be synthesized

as short single-stranded oligonucleotides, their small size together with their potency/stability provided by the LNA-modifications make delivery possible without vehicle-systems. For the rest of this review we will summarize miRNA drugs currently in clinical trials or likely to enter clinical trials in the near future.

Miravirsen (Santaris Pharma)

Miravirsen (or SPC3649) is a LNA-modified oligonucleotide designed to inhibit miR-122 developed by Danish firm Santaris Pharma A/S⁷⁹. This liver specific and highly abundant miRNA accounts for more than 70% of all miRNAs in the liver and has been shown to be crucial for the functional infection of Hepatitis C virus (HCV). The exact mechanism of how this miRNA facilitates viral replication is not clearly understood. It is suggested that interaction between miR-122 and two seed-sites in the 5' noncoding region of HCV induces viral transcripts, giving this miRNA a non-classical function of inducing rather than inhibiting the function of its target⁸⁰.

Treatment of chronic Hepatitis C-infected chimpanzees with miravirsen led to suppression of HCV without any obvious side-effects. Further, liver transcriptome analysis revealed 259 mRNAs containing full 8mer-seed miR-122 predicted binding sites to display increased expression following treatment, thus indicating such transcripts to be targeted by endogenous miR-122 in Hepatitis C. It is likely that some of those transcripts play additional therapeutic roles. Total serum cholesterol was reduced with downregulation of cholesterol metabolism genes⁷⁹.

Since reporting their non-human primate study, miravirsen has gone through two phase I clinical trials, successfully proving that the drug is safe even in humans (NCT00688012, NCT00979927), and a Phase IIa clinical trial (NCT01200420) (Figure 1). This Phase IIa trial enrolled 38 patients with treatment-naïve chronic HCV

infection to monitor safety, tolerability, pharmacokinetics and efficacy on HCV viral titer. Multiple dosage of miravirsin administered subcutaneously to patients gave promising outcomes with a mean reduction of HCV RNA levels by two to three logarithmic levels. Further, almost half of the patients treated by the highest dose displayed undetectable levels of HCV RNA within 4 weeks. These results are encouraging and highlight miravirsin as a potential future replacement therapy for patients with chronic HCV infection.

Discovering the general potential of miRNA inhibitory therapeutics using LNA-based or similar platforms for a wide range of diseases is a major task for the future. One might imagine that the tissue specificity of miR-122, its high abundance in this tissue compared to other miRNAs and the general convenience of delivering drugs systemically to the liver makes inhibition in this case easier than most other miRNAs. Despite such possible concerns, the development of miravirsin by Santaris Pharma provides a landmark breakthrough for miRNA based therapeutics.

Anti-miRs (Regulus Therapeutics)

Regulus Therapeutics is a San Diego based company with a wide-range program focusing on targeting endogenous miRNAs through inhibitory oligonucleotides in hepatocellular carcinoma, kidney fibrosis, atherosclerosis, HCV infection and glioblastoma. To keep such a platform running, Regulus has partnered with the corporate giants Sanofi, GlaxoSmithKline and AstraZeneca. In 2010, Regulus signed a deal with GlaxoSmithKline for the development of a miR-122 inhibitor drug, thus providing direct competition with Santaris Pharma (see above).

None of Regulus' programs have reached clinical phases but substantial preclinical work has been completed including a non-human primate study of inhibiting miR-33a/b for the treatment of atherosclerosis⁸¹ (Figure 1). miR-33b and miR-33a are encoded in the introns of the transcription factor loci *SREBP1* and *SREBP2*, respectively, and are involved in the regulation of cholesterol and fatty acid homeostasis. This puts them as potential targets for treatment of cardiovascular diseases⁸¹. By treating African Green monkeys subcutaneously with 2'-fluoro-methoxyethyl-phosphorothioate modified antisense-miR-33 oligonucleotides (anti-miR-33), the study demonstrated a decrease in very-low-density lipoprotein (VLDL)-triglycerides and an increase in high-density lipoprotein (HDL)⁸¹. Mechanistically, there was a significantly reduced repression of miR-33 predicted target genes and primates went through treatment without displaying significant side-effects⁸¹.

Using another anti-miR (anti-miR-21), Regulus convincingly demonstrated that their technology could be used for the inhibition of migration/invasion of glioma in mice⁸², attenuating cardiac dysfunction in a mouse model of cardiac disease⁸³ and executing an anti-fibrotic response in mice exposed to kidney injury⁸⁴. Results from additional mouse-models exposed to Regulus-developed anti-miRs include suppression of lung-metastasis originating from breast tumors (anti-miR-10b)⁸⁵, inhibition of neuroblastoma (anti-miR-380-5p)⁸⁶, antagonizing liver metastasis sourcing from melanoma (anti-miR-182)⁸⁷ and improved glucose homeostasis and insulin sensitivity (anti-miR-103/107)⁸⁸ (Figure 1).

MicroRNA replacement therapy, MRX34 (Mirna Therapeutics)

In contrast to the companies described above, Mirna Therapeutics is not focusing their platform around the inhibition of endogenous miRNAs but instead on introducing synthetic miRNA mimics as replacement therapies. The idea is to restore expression of certain miRNAs in tumors to a comparable level to surrounding healthy tissues. The platform of Mirna Therapeutics consists of eight drug candidates, solely focused on addressing tumor suppressive roles of miRNAs. Three out of eight candidate mimics represent miRNAs demonstrated by numerous publications to display tumor suppressive properties (miR-34, let-7 and miR-16). Their lead candidate, MRX34, a miR-34a mimic compound, will probably be the first miRNA replacement compound to reach clinical trials (Figure 1). At the time of writing this review, Mirna Therapeutics is recruiting participants to a Phase I study of MRX34 (NCT01829971).

miR-34a represents one of the most documented tumor suppression-associated miRNAs, being a transcriptional product of the transcription factor and genome guardian p53⁸⁹. Pre-clinical work by Mirna Therapeutics has demonstrated potent anti-tumor effects by introducing miR-34a mimics into a variety of mice cancer models^{90,91}.

The usage of miRNA mimics for systemic delivery is challenging compared to anti-miR drugs. miRNA mimics need to be double-stranded in order to be processed correctly by the cellular RNAi-machinery and therefore cannot be administered "naked". Successful delivery therefore requires complex delivery vehicles mimicking physiological settings where miRNAs reside in microvesicles or exosomes. For MRX34, Mirna therapeutics has developed custom nanoparticle liposomes. According to company information (MIRNA THERAPEUTICS) these liposomes increase stability, enhance delivery and prevent immune response effects. Extensive pre-clinical testing of MRX34 in mouse models of hepatocellular carcinoma using liposomes provided promising outcomes and the upcoming clinical trial is recruiting patients with non resectable primary liver cancer or metastatic cancer with liver involvement (NCT01829971).

Anti-miRs (miRagen Therapeutics)

miRagen Therapeutics is an American company founded in 2007 for the development of miRNA based drugs treating cardiac and muscular diseases. A partner alliance with Danish Santaris Pharma gives miRagen commercial rights to the LNA-based technique developed by Santaris. miRagen has programs covering both anti-miR and miRNA mimics. The anti-miR program contains candidate drugs relatively close to clinical stages (Figure 1). This platform includes three drugs: MGN-9103, MGN-1374 and MGN-4893.

The lead drug, MGN-9103, targets miR-208 with implications for the treatment of chronic heart failure. miR-208 is a heart specific miRNA located in an intron of the alphaMHC gene, which has been demonstrated to be required for cardiac hypertrophy, myosin switching and fibrosis in response to stress⁹². Such cardiac remodeling was blocked by treating rats subcutaneously with LNA-based anti-miR-208 during hypertension induced heart failure⁹³. Interestingly, MGN-9103 was also recently suggested to play beneficial roles in a mouse model of diabetes/obesity⁹⁴.

MGN-1374 targets miR-15 and miR-195, miRNAs shown to be upregulated in mouse hearts shortly after birth. Upregulation of miR-15 and miR-195 executes a postnatal cell cycle arrest in the heart generation after myocardial infarction⁹⁵. By inhibiting these miRNAs using their LNA-based MGN-1374, miRagen enables post-myocardial infarction remodeling. Such remodeling enhanced heart function and induced cardiomyocyte proliferation in mice and pigs⁹⁶.

MGN-4893, targets miR-451, a miRNA required for the expansion of red blood cells. Inhibition of miR-451 in mice using anti-miR-451 blocked erythrocyte differentiation suggesting that such inhibitors could be useful for the treatment of disorders leading to abnormal red blood cell production such as polycythemia vera⁹⁷.

Concluding remarks

MicroRNAs have come a long way since the initial discoveries two decades ago. Their emerging potential as biomarkers in clinical

diagnostics as well as modulators for the treatment of a variety of diseases is truly exciting. In the near future it will become clearer as to whether they have the power to become established as new molecular diagnostic benchmarks and whether microRNA-based therapy can compete with that of selective protein inhibitors.

Author contributions

PH wrote the initial draft of the article, GB reviewed and wrote the final draft.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

- Wightman B, Ha J, Ruvkun G: **Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*.** *Cell*. 1993; 75(5): 855–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lee RC, Feinbaum RL, Ambros V: **The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14.** *Cell*. 1993; 75(5): 843–54.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lee RC, Ambros V: **An extensive class of small RNAs in *Caenorhabditis elegans*.** *Science*. 2001; 294(5543): 862–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lau NC, Lim LP, Weinstein EG, et al.: **An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*.** *Science*. 2001; 294(5543): 858–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lagos-Quintana M, Rauhut R, Lendeckel W, et al.: **Identification of novel genes coding for small expressed RNAs.** *Science*. 2001; 294(5543): 853–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Griffiths-Jones S, Saini HK, van Dongen S, et al.: **miRBase: tools for microRNA genomics.** *Nucleic Acids Res*. 2008; 36(Database issue): D154–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ambros V, Bartel B, Bartel DP, et al.: **A uniform system for microRNA annotation.** *RNA*. 2003; 9(3): 277–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kim VN, Han J, Siomi MC: **Biogenesis of small RNAs in animals.** *Nat Rev Mol Cell Biol*. 2009; 10(2): 126–39.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Baskerville S, Bartel DP: **Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes.** *RNA*. 2005; 11(3): 241–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Landgraf P, Rusu M, Sheridan R, et al.: **A mammalian microRNA expression atlas based on small RNA library sequencing.** *Cell*. 2007; 129(7): 1401–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ozsolak F, Poling LL, Wang Z, et al.: **Chromatin structure analyses identify miRNA promoters.** *Genes Dev*. 2008; 22(22): 3172–83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lee Y, Kim M, Han J, et al.: **MicroRNA genes are transcribed by RNA polymerase II.** *EMBO J*. 2004; 23(20): 4051–60.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Cai X, Hagedorn CH, Cullen BR: **Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs.** *RNA*. 2004; 10(12): 1957–66.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Saini HK, Griffiths-Jones S, Enright AJ: **Genomic analysis of human microRNA transcripts.** *Proc Natl Acad Sci U S A*. 2007; 104(45): 17719–24.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Borchert GM, Lanier W, Davidson BL: **RNA polymerase III transcribes human microRNAs.** *Nat Struct Mol Biol*. 2006; 13(12): 1097–101.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Marson A, Levine SS, Cole MF, et al.: **Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells.** *Cell*. 2008; 134(3): 521–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pawlicki JM, Steitz JA: **Primary microRNA transcript retention at sites of transcription leads to enhanced microRNA production.** *J Cell Biol*. 2008; 182(1): 61–76.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Park JE, Heo I, Tian Y, et al.: **Dicer recognizes the 5' end of RNA for efficient and accurate processing.** *Nature*. 2011; 475(7355): 201–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Guo H, Ingolia NT, Weissman JS, et al.: **Mammalian microRNAs predominantly act to decrease target mRNA levels.** *Nature*. 2010; 466(7308): 835–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bartel DP: **MicroRNAs: target recognition and regulatory functions.** *Cell*. 2009; 136(2): 215–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lu J, Getz G, Miska EA, et al.: **MicroRNA expression profiles classify human cancers.** *Nature*. 2005; 435(7043): 834–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Sandberg R, Neilson JR, Sarma A, et al.: **Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites.** *Science*. 2008; 320(5883): 1643–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chin LJ, Ratner E, Leng S, et al.: **A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk.** *Cancer Res*. 2008; 68(20): 8535–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Karginov FV, Conaco C, Xuan Z, et al.: **A biochemical approach to identifying microRNA targets.** *Proc Natl Acad Sci U S A*. 2007; 104(49): 19291–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lal A, Thomas MP, Altschuler G, et al.: **Capture of microRNA-bound mRNAs identifies the tumor suppressor miR-34a as a regulator of growth factor signaling.** *PLoS Genet*. 2011; 7(11): e1002363.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chi SW, Zang JB, Mele A, et al.: **Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps.** *Nature*. 2009; 460(7254): 479–86.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Selbach M, Schwanhauss B, Thierfelder N, et al.: **Widespread changes in protein synthesis induced by microRNAs.** *Nature*. 2008; 455(7209): 58–63.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Baek D, Villen J, Shin C, et al.: **The impact of microRNAs on protein output.** *Nature*. 2008; 455(7209): 64–71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nelson PT, Baldwin DA, Searce LM, et al.: **Microarray-based, high-throughput**

- gene expression profiling of microRNAs.** *Nat Methods.* 2004; 1(2): 155–61.
[PubMed Abstract](#) | [Publisher Full Text](#)
30. Mitchell PS, Parkin RK, Kroh EM, *et al.*: **Circulating microRNAs as stable blood-based markers for cancer detection.** *Proc Natl Acad Sci U S A.* 2008; 105(30): 10513–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 31. Volinia S, Calin GA, Liu CG, *et al.*: **A microRNA expression signature of human solid tumors defines cancer gene targets.** *Proc Natl Acad Sci U S A.* 2006; 103(7): 2257–61.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 32. Blenkiron C, Goldstein LD, Thorne NP, *et al.*: **MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype.** *Genome Biol.* 2007; 8(10): R214.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 33. Sempere LF, Christensen M, Silahatoglu A, *et al.*: **Altered MicroRNA expression confined to specific epithelial cell subpopulations in breast cancer.** *Cancer Res.* 2007; 67(24): 11612–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
 34. Takamizawa J, Konishi H, Yanagisawa K, *et al.*: **Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival.** *Cancer Res.* 2004; 64(11): 3753–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
 35. Calin GA, Ferracin M, Cimmino A, *et al.*: **A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia.** *N Engl J Med.* 2005; 353(17): 1793–801.
[PubMed Abstract](#) | [Publisher Full Text](#)
 36. van Rooij E, Sutherland LB, Liu N, *et al.*: **A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure.** *Proc Natl Acad Sci U S A.* 2006; 103(48): 18255–60.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 37. Eisenberg I, Eran A, Nishino I, *et al.*: **Distinctive patterns of microRNA expression in primary muscular disorders.** *Proc Natl Acad Sci U S A.* 2007; 104(43): 17016–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 38. Hebert SS, De Strooper B: **Alterations of the microRNA network cause neurodegenerative disease.** *Trends Neurosci.* 2009; 32(4): 199–206.
[PubMed Abstract](#) | [Publisher Full Text](#)
 39. Lu M, Zhang Q, Deng M, *et al.*: **An analysis of human microRNA and disease associations.** *PLoS One.* 2008; 3(10): e3420.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 40. Schwarzenbach H, Hoon DS, Pantel K: **Cell-free nucleic acids as biomarkers in cancer patients.** *Nat Rev Cancer.* 2011; 11(6): 426–37.
[PubMed Abstract](#) | [Publisher Full Text](#)
 41. Lawrie CH, Gal S, Dunlop HM, *et al.*: **Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma.** *Br J Haematol.* 2008; 141(5): 672–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
 42. Heneghan HM, Miller N, Kelly R, *et al.*: **Systemic miRNA-195 differentiates breast cancer from other malignancies and is a potential biomarker for detecting noninvasive and early stage disease.** *Oncologist.* 2010; 15(7): 673–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. Huang Z, Huang D, Ni S, *et al.*: **Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer.** *Int J Cancer.* 2010; 127(1): 118–26.
[PubMed Abstract](#) | [Publisher Full Text](#)
 44. Xing L, Todd NW, Yu L, *et al.*: **Early detection of squamous cell lung cancer in sputum by a panel of microRNA markers.** *Mod Pathol.* 2010; 23(8): 1157–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
 45. Boeri M, Verri C, Conte D, *et al.*: **MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer.** *Proc Natl Acad Sci U S A.* 2011; 108(9): 3713–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 46. Keller A, Leidinger P, Bauer A, *et al.*: **Toward the blood-borne miRNome of human diseases.** *Nat Methods.* 2011; 8(10): 841–3.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. Petriv OI, Kuchenbauer F, Delaney AD, *et al.*: **Comprehensive microRNA expression profiling of the hematopoietic hierarchy.** *Proc Natl Acad Sci U S A.* 2010; 107(35): 15443–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 48. Rechavi O, Erlich Y, Amram H, *et al.*: **Cell contact-dependent acquisition of cellular and viral nonautonomously encoded small RNAs.** *Genes Dev.* 2009; 23(16): 1971–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 49. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, *et al.*: **Functional delivery of viral miRNAs via exosomes.** *Proc Natl Acad Sci U S A.* 2010; 107(14): 6328–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 50. Yuan A, Farber EL, Rapoport AL, *et al.*: **Transfer of microRNAs by embryonic stem cell microvesicles.** *PLoS One.* 2009; 4(3): e4722.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 51. Kosaka N, Iguchi H, Yoshioka Y, *et al.*: **Secretory mechanisms and intercellular transfer of microRNAs in living cells.** *J Biol Chem.* 2010; 285(23): 17442–52.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 52. Hanke M, Hoefig K, Merz H, *et al.*: **A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer.** *Urol Oncol.* 2010; 28(6): 655–61.
[PubMed Abstract](#) | [Publisher Full Text](#)
 53. Michael A, Bajracharya SD, Yuen PS, *et al.*: **Exosomes from human saliva as a source of microRNA biomarkers.** *Oral Dis.* 2010; 16(1): 34–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 54. Park NJ, Zhou H, Elashoff D, *et al.*: **Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection.** an official journal of the American Association for Cancer Research. *Clin Cancer Res.* 2009; 15(17): 5473–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 55. Iorio MV, Croce CM: **MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review.** *EMBO Mol Med.* 2012; 4(3): 143–59.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 56. Chen C, Ridzon DA, Broomer AJ, *et al.*: **Real-time quantification of microRNAs by stem-loop RT-PCR.** *Nucleic Acids Res.* 2005; 33(20): e179.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 57. de Planell-Saguer M, Rodicio MC, Mourelatos Z: **Rapid *in situ* codetection of noncoding RNAs and proteins in cells and formalin-fixed paraffin-embedded tissue sections without protease treatment.** *Nat Protoc.* 2010; 5(6): 1061–73.
[PubMed Abstract](#) | [Publisher Full Text](#)
 58. Ramaswamy S, Tamayo P, Rifkin R, *et al.*: **Multiclass cancer diagnosis using tumor gene expression signatures.** *Proc Natl Acad Sci U S A.* 2001; 98(26): 15149–54.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 59. Rosenfeld N, Aharonov R, Meiri E, *et al.*: **MicroRNAs accurately identify cancer tissue origin.** *Nat Biotechnol.* 2008; 26(4): 462–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 60. Varadhachary GR, Spector Y, Abbuzzese JL, *et al.*: **Prospective gene signature study using microRNA to identify the tissue of origin in patients with carcinoma of unknown primary.** *Clin Cancer Res.* 2011; 17(12): 4063–70.
[PubMed Abstract](#) | [Publisher Full Text](#)
 61. Mueller WC, Spector Y, Edmonston TB, *et al.*: **Accurate classification of metastatic brain tumors using a novel microRNA-based test.** *Oncologist.* 2011; 16(2): 165–74.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 62. Meiri E, Mueller WC, Rosenwald S, *et al.*: **A second-generation microRNA-based assay for diagnosing tumor tissue origin.** *Oncologist.* 2012; 17(6): 801–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 63. Gilad S, Lithwick-Yanai G, Barshack I, *et al.*: **Classification of the four main types of lung cancer using a microRNA-based diagnostic assay.** *J Mol Diagn.* 2012; 14(5): 510–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
 64. Lebanony D, Benjamin H, Gilad S, *et al.*: **Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma.** *J Clin Oncol.* 2009; 27(12): 2030–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
 65. Benjamin H, Lebanony D, Rosenwald S, *et al.*: **A diagnostic assay based on microRNA expression accurately identifies malignant pleural mesothelioma.** *J Mol Diagn.* 2010; 12(6): 771–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 66. Fridman E, Dotan Z, Barshack I, *et al.*: **Accurate molecular classification of renal tumors using microRNA expression.** *J Mol Diagn.* 2010; 12(5): 687–96.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 67. Ryan BM, Robles AI, Harris CC: **Genetic variation in microRNA networks: the implications for cancer research.** *Nat Rev Cancer.* 2010; 10(6): 389–402.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 68. Hu Z, Liang J, Wang Z, *et al.*: **Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women.** *Hum Mutat.* 2009; 30(1): 79–84.
[PubMed Abstract](#) | [Publisher Full Text](#)
 69. Christensen BC, Avissar-Whiting M, Ouellet LG, *et al.*: **Mature microRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer.** *Clin Cancer Res.* 2010; 16(14): 3713–20.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 70. Shen J, Ambrosone CB, DiCioccio RA, *et al.*: **A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis.** *Carcinogenesis.* 2008; 29(10): 1963–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
 71. Horikawa Y, Wood CG, Yang H, *et al.*: **Single nucleotide polymorphisms of microRNA machinery genes modify the risk of renal cell carcinoma.** *Clin Cancer Res.* 2008; 14(23): 7956–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 72. He H, Jazdzewski K, Li W, *et al.*: **The role of microRNA genes in papillary thyroid carcinoma.** *Proc Natl Acad Sci U S A.* 2005; 102(52): 19075–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 73. Liu Z, Wei S, Ma H, *et al.*: **A functional variant at the miR-184 binding site in TNFAIP2 and risk of squamous cell carcinoma of the head and neck.**

- Carcinogenesis*. 2011; **32**(11): 1668–74.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
74. 1000 Genomes Project ConsortiumAbecasis GR, Altshuler D, Auton A, *et al.*: **A map of human genome variation from population-scale sequencing**. *Nature*. 2010; **467**(7319): 1061–73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 75. Richardson K, Lai CQ, Parnell LD, *et al.*: **A genome-wide survey for SNPs altering microRNA seed sites identifies functional candidates in GWAS**. *BMC genomics*. 2011; **12**: 504.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 76. Burnett JC, Rossi JJ: **RNA-based therapeutics: current progress and future prospects**. *Chem Biol*. 2012; **19**(1): 60–71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 77. Shukla S, Sumaria CS, Pradeepkumar PI: **Exploring chemical modifications for siRNA therapeutics: a structural and functional outlook**. *Chem Med Chem*. 2010; **5**(3): 328–49.
[PubMed Abstract](#) | [Publisher Full Text](#)
 78. Siegwart DJ, Whitehead KA, Nuhn L, *et al.*: **Combinatorial synthesis of chemically diverse core-shell nanoparticles for intracellular delivery**. *Proc Natl Acad Sci U S A*. 2011; **108**(32): 12996–3001.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 79. Lanford RE, Hildebrandt-Eriksen ES, Petri A, *et al.*: **Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection**. *Science*. 2010; **327**(5962): 198–201.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 80. Jopling CL, Yi M, Lancaster AM, *et al.*: **Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA**. *Science*. 2005; **309**(5740): 1577–81.
[PubMed Abstract](#) | [Publisher Full Text](#)
 81. Rayner KJ, Esau CC, Hussain FN, *et al.*: **Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides**. *Nature*. 2011; **478**(7369): 404–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 82. Gabriely G, Wurdinger T, Kesari S, *et al.*: **MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators**. *Mol Cell Biol*. 2008; **28**(17): 5369–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 83. Thum T, Gross C, Fiedler J, *et al.*: **MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts**. *Nature*. 2008; **456**(7224): 980–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
 84. Chau BN, Xin C, Hartner J, *et al.*: **MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways**. *Sci Transl Med*. 2012; **4**(121): 121ra18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 85. Ma L, Reinhardt F, Pan E, *et al.*: **Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model**. *Nat Biotechnol*. 2010; **28**(4): 341–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 86. Swarbrick A, Woods SL, Shaw A, *et al.*: **miR-380-5p represses p53 to control cellular survival and is associated with poor outcome in MYCN-amplified neuroblastoma**. *Nat Med*. 2010; **16**(10): 1134–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 87. Huynh C, Segura MF, Gaziel-Sovran A, *et al.*: **Efficient *in vivo* microRNA targeting of liver metastasis**. *Oncogene*. 2011; **30**(12): 1481–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 88. Trajkovski M, Hausser J, Soutschek J, *et al.*: **MicroRNAs 103 and 107 regulate insulin sensitivity**. *Nature*. 2011; **474**(7353): 649–53.
[PubMed Abstract](#) | [Publisher Full Text](#)
 89. He L, He X, Lim LP, *et al.*: **A microRNA component of the p53 tumour suppressor network**. *Nature*. 2007; **447**(7148): 1130–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
 90. Liu C, Kelnar K, Liu B, *et al.*: **The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44**. *Nat Med*. 2011; **17**(2): 211–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 91. Trang P, Wiggins JF, Daige CL, *et al.*: **Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice**. *Mol Ther*. 2011; **19**(6): 1116–22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 92. van Rooij E, Sutherland LB, Qi X, *et al.*: **Control of stress-dependent cardiac growth and gene expression by a microRNA**. *Science*. 2007; **316**(5824): 575–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 93. Montgomery RL, Hullinger TG, Semus HM, *et al.*: **Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure**. *Circulation*. 2011; **124**(14): 1537–47.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 94. Grueter CE, van Rooij E, Johnson BA, *et al.*: **A cardiac microRNA governs systemic energy homeostasis by regulation of MED13**. *Cell*. 2012; **149**(3): 671–83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 95. Porrello ER, Mahmoud AI, Simpson E, *et al.*: **Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family**. *Proc Natl Acad Sci U S A*. 2013; **110**(1): 187–92.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 96. Hullinger TG, Montgomery RL, Seto AG, *et al.*: **Inhibition of miR-15 protects against cardiac ischemic injury**. *Circ Res*. 2012; **110**(1): 71–81.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 97. Patrick DM, Zhang CC, Tao Y, *et al.*: **Defective erythroid differentiation in miR-451 mutant mice mediated by 14-3-3zeta**. *Genes Dev*. 2010; **24**(15): 1614–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Current Peer Review Status:  

Version 1

Reviewer Report 24 June 2013

<https://doi.org/10.5256/f1000research.1453.r1021>

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Hydbring and Badalian-Very summarize in this review, the current status in the potential development of clinical applications based on miRNAs' biology. The article gives an interesting historical and scientific perspective on a field that has only recently boomed; focusing mostly on the two main products in the pipeline of several biotech companies (in Europe and USA) which work with miRNAs-based agents, disease diagnostics and therapeutics. Interestingly, not only the specific agents that are being produced are mentioned, but also clever insights in the important cellular pathways regulated by key miRNAs are briefly discussed.

Minor points to consider in subsequent versions:

1. Page 2; paragraph '*Genomic location and transcription of microRNAs*': the concept of miRNA clusters and precursors could be a bit better explained.
2. Page 2; paragraph '*Genomic location and transcription of microRNAs*': when discussing the paper by the laboratory of Richard Young (reference 16); I think it is important to mention that that particular study refers to stem cells.
3. Page 2; paragraph '*Processing of microRNAs*': "Argonate" should be replaced by "Argonaute".
4. Page 3; paragraph '*MicroRNAs in disease diagnostics*': are miR-15a and 16-1 two different miRNAs? I suggest mentioning them as: miR-15a and miR-16-1 and not using a slash sign (/) between them.
5. Page 4; paragraph '*Circulating microRNAs*': I am a bit bothered by the description of multiple sclerosis (MS) only as an autoimmune disease. Without being an expert in the field, I believe that there are other hypotheses related to the etiology of MS.
6. Page 5; paragraph '*Clinical microRNA diagnostics*': Does 'hsa' in hsa-miR-205 mean

something?

7. Page 5; paragraph '*Clinical microRNA diagnostics*': the authors mention the company Asuragen, Austin, TX, USA but they do not really say anything about their products. I suggest to either remove the reference to that company or to include their current pipeline efforts.
8. Page 6; paragraph '*MicroRNAs in therapeutics*': in the first paragraph the authors suggest that miRNAs-based therapeutics should be able to be applied with "minimal side-effects". Since one miRNA can affect a whole gene program, I found this a bit counterintuitive; I was wondering if any data has been published to support that statement. Also, in the same paragraph, the authors compare miRNAs to protein inhibitors, which are described as more specific and/or selective. I think there are now good indications to think that protein inhibitors are not always that specific and/or selective and that such a property actually could be important for their evidenced therapeutic effects.
9. Page 6; paragraph '*MicroRNAs in therapeutics*': I think the concept of "antagomir" is an important one and could be better highlighted in the text.
10. Throughout the text (pages 3, 5, 6, and 7): I am a bit bothered by separating the word "miRNA" or "miRNAs" at the end of a sentence in the following way: "miR-NA" or "miR-NAs". It is a bit confusing considering the particular nomenclature used for miRNAs. That was probably done during the formatting and editing step of the paper.
11. I was wondering if the authors could develop a bit more the general concept that seems to indicate that in disease (and in particular in cancer) the expression and levels of miRNAs are in general downregulated. Maybe some papers have been published about this phenomenon?

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 15 Aug 2013

Per Hydbring, Dana-Farber Cancer Institute and Harvard Medical School, Boston MA, USA

We thank reviewer Dr. Gustavo Gutierrez Gonzalez, for very valuable comments helping to improve our manuscript. We have adjusted our text according to the comments from Dr. Gutierrez Gonzalez. For clarification, we include a bullet-point response to each comment below.

1. In response to this comment we have added a few words in this paragraph. Further, we added a sentence in the previous paragraph "MicroRNA discovery".
2. We have clarified in this paragraph that the study refers to stem cells.

3. We thank the reviewer for pointing this out. This typo has been adjusted.
4. miR-15a and 16-1 originate from the same polycistronic transcript and have identical seed sequences. However, as the reviewer point out, they are still two separate mature miRNAs. We have therefore adjusted the text according to the reviewer's suggestion to avoid confusion.
5. We agree with the reviewer of this misleading description and have adjusted the text accordingly.
6. This means "homo sapiens" but to avoid confusion we have removed it from miR-205.
7. In accordance to the reviewer's suggestion we have removed this company reference.
8. Since there is currently only one miRNA drug in clinical trials, there is little direct evidence of that miRNAs can be applied with minimal side-effects. In support of our hypothesis, their mechanism of action is tuning expression rather than blunting their targets which reasonably should be less detrimental to healthy tissues. We have added one sentence in this paragraph to clarify our hypothesis. We further agree with the reviewer that a lot of enzymatic protein inhibitors could be more selective. However, the purpose of these drugs is selectivity. In contrast, miRNA drugs are developed with the idea of controlling multiple gene-components in the same or overlapping signaling-pathways. Such gene-products are not limited to proteins with enzymatic activity but could include any deregulated genes or proteins in a given disease.
9. We agree with the reviewer and have made slight adjustments in the text to better highlight this concept.
10. This has been an editing/formatting mistake. In our original manuscript there are no separations of the word "miRNA" or "miRNAs".
11. We describe downregulation of miRNAs in the section "MicroRNAs in disease diagnostics" referring to a few publications (25, 35-39). To better clarify a general impact of reduced miRNA expression in cancers we included an additional reference (40), describing a conditional knockout mice model for miRNA biogenesis leading to reduced survival in affected mice.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 June 2013

<https://doi.org/10.5256/f1000research.1453.r1008>

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**Florian Karreth**

Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA

This review article by Hydbing and Badalian-Very summarizes our current knowledge of microRNAs and outlines their clinical value. The authors provide a comprehensive overview of microRNA biology and target prediction and discuss the utility of miRNAs as prognostic and diagnostic markers and therapeutic targets. Finally, Hydbing and Badalian-Very highlight the various strategies adopted by leading biotechnology companies and the state of their current clinical trials.

I have two minor suggestions regarding the biology of miRNAs that the authors may wish to include in their manuscript:

1. A recent paper by paper by Tollervey and colleagues ([Helwak *et al.*, Cell 2013](#)) describes that a large fraction of miRNAs bind their targets independent of seed match complementarity at nucleotides 2-7. This observation greatly affects our ability to accurately predict miRNA targets using existing algorithms.
2. Besides the 3'UTR, miRNAs have been demonstrated to target the 5'UTR and coding sequence of mRNAs as well as other RNA species such as lncRNAs, pseudogenes, rRNAs and tRNAs.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 15 Aug 2013

Per Hydbing, Dana-Farber Cancer Institute and Harvard Medical School, Boston MA, USA

We thank reviewer Dr. Florian Karreth for very valuable comments helping to improve our manuscript. We have adjusted our text according to the comments from Dr. Karreth. For clarification, we include a bullet-point response to each comment below.

1. We thank the reviewer for pointing out this paper which brings the understanding of microRNA targeting to a higher level. We have included this reference and commented on it in the text (paragraph "MicroRNA functional targeting").
2. The reviewer makes a very important point and to cover some of these mechanisms we have added three references and commented shortly on them in the text (paragraph "MicroRNA functional targeting"). However, since our main focus is on the clinical aspects of microRNAs we believe a deeper description of non-canonical

microRNA targeting is out of scope for this review.

Competing Interests: No competing interests were disclosed.

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