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Genomics Proteomics Bioinformatics 10 (2012) 246–253

GENOMICS PROTEOMICS & BIOINFORMATICS

www.elsevier.com/locate/gpb

Review

MicroRNAs in Common Human Diseases

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Received 5 May 2012; revised 28 June 2012; accepted 10 July 2012
Available online 29 September 2012

Abstract

MicroRNAs (miRNAs) are a class of short non-coding RNA molecules that have attracted tremendous attention from the biological and biomedical research communities over the past decade. With over 1900 miRNAs discovered in humans to date, many of them have already been implicated in common human disorders. Facilitated by high-throughput genomics and bioinformatics in conjunction with traditional molecular biology techniques and animal models, miRNA research is now positioned to make the transition from laboratories to clinics to deliver profound benefits to public health. Herein, we overview the progress of miRNA research related to human diseases, as well as the potential for miRNA to becoming the next generation of diagnostics and therapeutics.

Keywords: MicroRNA; Human diseases; Diagnostics; Therapeutics; Biomarker

A historical overview of microRNA research

MicroRNAs (miRNAs) are a class of recently identified non-coding RNA molecules that play an essential role in gene expression regulation at post-transcriptional levels [1]. With the first miRNA, lin-4, discovered in *Caenorhabditis elegans* in 1993 via forward genetics [2], the second *C. elegans* miRNA, let-7, was not identified by the same approach until seven years later [3]. This time gap highlights not only the inefficiency of forward genetics and standard molecular biology techniques to discover miRNAs, but also the lack of enthusiasm among researchers who previously suspected that miRNA was merely a worm-specific phenomenon. However, the field of miRNA research has since flourished with over 17,000 miRNAs discovered to date in 142 species, including more than 1900 in humans [4]. The key word "miRNA" currently pulls more than 16,000 publications from PubMed, and the first miRNA-targeted drug has now entered a phase II clinical trial (http://www.ClinicalTrials.gov), demonstrating early promise. In retrospect, the timing of miRNA research evolution was particularly interesting as it echoed the time frame of the Human Genome Project (HGP) and many other whole-genome sequencing projects completed over the past decade. The completion of these projects has impacted the field of miRNA research in profound ways.

The fruitful expansion of miRNA research was triggered by the identification and functional characterization of let-7 [3]. When Ruvkun et al. demonstrated that the let-7 sequence was highly conserved across the evolutionary spectrum [5], biologists started to realize that this tiny RNA molecule may have a big role to play in humans as well [6]. Before long, three competing laboratories made *de novo* identifications of dozens of single-stranded RNA molecules approximately 22 nt in length by the combination of an improved cloning method and bioinformatics, a novel approach at the time [7–9]. The method of *de novo* identification was rather successful, leading to most miRNA discoveries before 2006, including more than 300 in humans. More importantly, it revealed the intrinsic characteristics of miRNA as a class, such as the secondary structure of miRNA precursors, allowing new miRNAs to be computationally identified. However, the *de novo* identification method came with a few limitations. It was difficult to clone miRNAs expressed at low levels or with certain sequence compositions and post-transcriptional

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http://dx.doi.org/10.1016/j.gpb.2012.07.005

modifications [10,11]. Nevertheless, these limitations could be bypassed through *in silico* prediction. With the completion of many whole genome sequencing projects [12–15], thousands of new miRNA species were now identifiable by computational prediction [4,16]. Taking a variety of factors into consideration, such as sequence conservation and thermodynamic stability of secondary structure, researchers were now able to identify new miRNA species that failed to be discovered by cloning approaches [17]. To date, the vast majority of known miRNA species have been discovered by bioinformatics and their sequences can be found in the Sanger miRNA registry (http://www.miRBase.com), an open access database for miRNA research [4].

The intriguing story of miRNA cannot be fully revealed without identifying miRNA targets in the context of biological processes. Through painstaking characterization of miRNA biogenesis and functional pathways [18], it is now clear that miRNAs repress the expression of cellular gene targets in a sequence-dependent manner. Specifically, the miRNA "seed", *i.e.*, the sequence between the 2nd and the 8th nt from the 5′ end, is essential in recognizing targets [19]. Facilitated by Dicer, an RNase III family member, the heteroduplex of miRNA and its target mRNA is integrated into the RNA-induced silencing complex (RISC). Mainly composed with the multi-functional catalytic protein, Argonaute, and a double stranded RNA binding protein, TRBP, responsible for recruiting Dicer to Argonaute [20–23], RISC plays a central role in miRNA-mediated repression on gene expression [21]. The type of repression relies on the degree of sequence complementarity between seed and target sequences. Whereas partial complementarity may induce translation repression or target mRNA instability, perfect complementarity normally causes target mRNA destruction [24]. This target recognition mechanism allows for *in silico* methods of target prediction by aligning miRNA sequences with entire genomes in searching for potential miRNA binding sites. Adopting similar algorithms, a few groups have developed open access target prediction software with minor variations, such as miRBase, PicTar, TargetScan and miRanda [25–30], *etc*. Conversely, researchers have been trying to use high-throughput genomic approaches, such as oligonucleotide microarrays facilitated by bioinformatics, to experimentally identify targets [31–33]. Evidence suggests that RNA destabilization is the predominant mechanism mediated by miRNA in mammals, making these methods particularly useful for identifying strong miRNA complementarities with marked effects [34]. Specifically, by introducing a miRNA of interest into cultured mammalian cells, the expression changes of predicted targets are monitored in real-time [31]. Sequence alignment of the artificially over-expressed miRNA and the down-regulated mRNA would further suggest a direct regulation or off-target effect. Both *in silico* prediction and target expression profiling suggest that the regulatory relationships of miRNAs and their targets are complex. Because of short seed sequences, multiple miRNAs may repress the expression of a specific gene simultaneously by targeting different sequence regions; likewise, a single miRNA may be able to regulate the expression of dozens or even hundreds of targets at the same time. Although it was initially believed that miRNA-mediated repression takes place exclusively in the cytoplasm, new evidence suggests that it may also occur in other cellular compartments such as mitochondria and nucleus [35,36]. The complexity of regulation underscores the necessity of combining traditional molecular biology with modern bioinformatic approaches to characterize the roles of miRNA more effectively.

miRNAs and human diseases

As discovery of human miRNAs increased, the research focus was gradually shifted towards functional characterization of miRNAs, particularly in the context of human diseases. The connection between miRNAs and disease was obvious. miRNA expression patterns are tissue-specific [37] and in many cases define the physiological nature of the cell [31]. The definitive evidence came from a report demonstrating that the gene expression profile of a non-neuron cell became more like that of a neuron when the neuron-specific miR-124 was artificially over-expressed within [31]. If the same premise holds true, certain miRNA expression patterns could be disease-specific and hold great prognostic value. In fact, a more comprehensive miRNA profiling study demonstrated that distinct miRNA expression patterns were specific to various types of cancers and were able to reflect the developmental lineage and differentiation state of tumors [38]. More specifically, many miRNAs were found to play key roles in vital biological processes such as cell division and death [39], cellular metabolism [40], intracellular signaling [41], immunity [42] and cell movement [43]. Therefore, aberrant miRNA expression should proportionately affect those critical processes, and as a result, lead to various pathological and occasionally malignant outcomes. Here, we overview miRNA-related studies focused on high-priority human diseases with insufficient treatment options (**Table 1**).

Cancers

Since the early stages of miRNA research, cancer has been the most prominent of human diseases with a clear role for miRNA regulation. The first evidence came from a study by Calin et al. in which they demonstrated a frequent deletion of miRNA genes *miR15* and *miR16* among 65% of B-cell chronic lymphocytic leukemia (B-CLL) patients [44]. Intriguingly, down-regulation of miR-15 and miR-16 expression was observed among B-CLL patients without the deletion, suggesting that the pathogenesis of B-CLL may be attributed to the intracellular abundance of two miRNAs. Encouraged by this finding, this group applied a systemic search on the complete human genome and established correlations of miRNAs with various cancers

Table 1 miRNAs associated with common human diseases

Disease		miRNA	Reference
Cancer	B-CLL	miR-15, miR-16	[44]
	Breast cancer	miR-125b, miR-145, miR-21, miR-155, miR-210	[46,56]
	Lung cancer	miR-155, let-7a	[47]
	Gastric cancer	miR-145	[54]
	Liver cancer	miR-29b	[57,58]
Viral diseases	HCV	miR-122, miR-155	[72,73,78]
	HIV-1	miR-28, miR-125b, miR-150, miR-223, miR-382	[75]
	Influenza virus	miR-21, miR-223	[76,77]
Immune-related diseases	Multiple sclerosis	miR-145, miR-34a, miR-155, miR-326	[80,81]
	Systemic lupus erythematosus	miR-146a	[82,83]
	Type II diabetes	miR-144, miR-146a, miR-150, miR-182, miR-103, miR-107	[84]
	Nonalcoholic fatty liver disease	miR-200a, miR-200b, miR-429, miR-122, miR-451, miR-27	[86]
	Non-alcoholic steatohepatitis	miR-29c, miR-34a, miR-155, miR-200b	[87]
Neurodegenerative diseases	Parkinson's disease	miR-30b, miR-30c, miR-26a, miR-133b, miR-184*, let-7	[90–92]
	Alzheimer's disease	miR-29b-1, miR-29a, miR-9	[94]

[45]. Subsequent expression profiling studies further demonstrated the correlation between aberrant miRNA expression patterns and increased occurrence of different types of cancers. Notably, the deregulation of miR-125b, miR-145, miR-21, and miR-155 expression was associated with the increased risk of breast cancer [46]. In addition, up-regulation of miR-155 and down-regulation of let-7a were correlated with poor survival of lung cancer patients [47], indicating an imbalance of cell death and proliferation during cancer development [48–50]. Intriguingly, miRNA expression patterns were also able to stage cancer progression [38], indicating that miRNA levels were not only useful in diagnosis but also potentially in prognosis of diseases. These cancer-related miRNAs were categorized into tumor suppressors and oncogenes due to their associations with opposite clinical outcomes with altered expressions. For example, miR-15, miR-16 and let-7 are known tumor suppressors while miR-21 and miR-155 serve as oncogenes [44,51,52].

The discovery of cancer-related miRNAs by expression profiling inspired mechanistic studies to implicate specific miRNAs in tumorigenesis pathways. miR-15 and miR-16 were found to repress the expression of anti-apoptotic gene *bcl-2* thereby promoting cell death in cancerous cells [52]. Likewise, let-7 family members demonstrate anti-cancer properties due to their ability to repress the expression of the oncogene, *ras* [53]. In contrast, miR-21 directly serves as an anti-apoptotic factor in glioblastomas and breast cancer [46,51]. Similarly, miR-155 interferes with the process of mismatch repair by repressing the expression of the MSH gene family members in colorectal cancer [54].

miRNAs also play key roles in tumor invasion and metastasis. miRNA expression profiling revealed the stepwise down-regulation of miR-145 levels with progression of primary gastric cancers and secondary metastases [55], as well as metastatic prostate cancer [56]. Similarly, increased expression of miR-210 was observed during the invasive transition of breast cancer [57]. While profiling studies establish disease correlations, mechanistic studies characterize the role of miRNAs in greater detail. For example, through the use of synthetic miRNA mimics, miR-7 and miR-29b were shown to suppress the metastasis of liver cancer by targeting PIK3CD [58] and MMP-2 [59], respectively. These cancer-related miRNAs are potentially useful for developing not only early diagnosis, but also novel anti-cancer strategies.

Viral diseases

Viruses are a group of pathogens with members causing not only severe, chronic diseases, but also some of the most deadly pandemics in human history. While miRNAs were being identified in eukaryotes, viral-encoded miRNAs were discovered in multiple virus species as well. The first viral-encoded miRNAs were cloned from a Burkitt's lymphoma cell line latently infected by Epstein–Barr virus (EBV), a DNA virus of the herpesvirus family [60]. Soon after, dozens of viral miRNAs were identified in polyoma virus[61], adenovirus [62], and several subtypes of the herpes viruses by cloning, bioinformatics, or combined approaches [63–65]. Some preliminary evidence even suggested that RNA viruses may also encode miRNAs in spite of small genome sizes [66–68]; however, these findings have not been verified independently [65,69].

Besides bearing viral miRNAs, alternatively, viruses are capable of regulating the expression of host cellular miRNAs for their own benefit. For example, unlike the Kaposi's sarcoma-associated herpes virus (KHSV) which encodes a viral miRNA, miR-K12-11, EBV is able to up-regulate the expression of cellular miR-155, an ortholog of miR-K12-11 [70]. Interestingly, these two miRNAs target the same set of cellular genes, indicating a similar function [71]. A more detailed study revealed that miR-155 may prevent EBV-infected cells from apoptotic death [72], a common strategy mediated by hosts to constrain viral infection. This demonstrates the potential consequences of a virus gaining control of cellular miRNA expression for its survival.

Although the expression of some cellular miRNAs is not directly regulated by viruses, maintenance of their intracellular level is pivotal for viral infection and replication. For example, high levels of liver-specific miR-122 expression is necessary for HCV replication both *in vitro* and *in vivo* [73,74], although viral infection and replication does not affect the expression of miR-122 [75]. On the contrary, copies of miR-28, miR-125b, miR-150, miR-223 and miR-382 are maintained at high levels in resting $CD4^+$ T cells, but significantly decreased in activated $CD4^+$ T cells, resulting in productive infection of HIV-1 in only the latter case [76]. These findings may help explain the tissue-specificity of virus infections and provide novel targets for anti-viral therapeutics.

Finally, miRNA expression changes may demonstrate how hosts respond to viral infections. For example, aberrant expression of a subset of cellular miRNAs was observed in lethal influenza virus infection, but not in non-lethal infection in animal models [77,78]. Specifically, miR-21 and miR-223 were strongly up-regulated in lethal infections of H1N1 pandemic influenza virus and H5N1 avian influenza virus in mice and macaques, respectively [77,78] while their expression was unchanged or only moderately up-regulated in animals infected with less pathogenic viruses. More recently, marked increase of miR-155 was seen in HCV-infected patients [79]. The up-regulation of miR-155 by HCV-infection may activate Wnt signaling pathway and contribute in part to HCV-induced hepatocarcinogenesis [79]. These variable miRNA expression patterns may be useful in guiding physicians to make treatment plans for patients infected by more or less virulent pathogens.

Immune-related diseases

Many common immune-related diseases, including multiple sclerosis (MS), systemic lupus erythematosus (SLE), type I/II diabetes, and nonalcoholic fatty *liver disease* (NAFLD), have shown established correlations with cellular miRNAs. Dozens of miRNA signatures were identified by comparing the miRNA expression profiles of relapsing-remitting MS and healthy controls [80]. Specifically, the expression of miR-145 alone was found to distinguish affected patients from healthy controls with high specificity and sensitivity. Increased expression of miR-34a, miR-155 and miR-326 was observed in MS lesions [81], with additional evidence indicating that high levels of miR-326 had a strong correlation with increased severity of MS [82]. In two independent studies involving hundreds of SLE patients and healthy controls, decreased expression of miR-146a demonstrated a strong correlation with increased risk for SLE among east Asian and European populations [83,84]. miRNA expression profiling has also identified type 2 diabetes-related miRNAs including miR-144, miR-146a, miR-150 and miR-182 [85]. In addition, miR-103 and miR-107 were shown to negatively regulate glucose homeostasis and insulin sensitivity in type 2 diabetes by targeting caveolin-1, a critical regulator of insulin receptor [86]. Increased expression of miR-200a, miR-200b and miR-429 and decreased expression of miR-122, miR-451 and miR-27 were associated with diet-induced NAFLD in rats [87]. Furthermore, abnormal expression of miR-29c, miR-34a, miR-155, and miR-200b were found in a mouse model of non-alcoholic steatohepatitis (NASH) [88], in addition to 23 more identified in tissues from NASH patients by miRNA microarrays [89].

Mechanistic studies revealed that miRNAs play critical roles in inflammation primarily by regulating the pathways associated with nuclear factor kappa beta (NF-κB), the central mediator of inflammatory response. The best characterized ones are miR-155 and miR-146, which were implicated in many immune-diseases [73,74,81,85,88]. In a negative feedback loop in which NF-κB activation up-regulates miR-146 expression, miR-146 subsequently down-regulates the expression of IRAK1 and TRAF6, two up-stream activators of NF-κB [42]. Similarly, increased expression of miR-155 by NF-κB could repress both IKK-β and IKK-ε, and prevent NF-κB from being constitutively activated [90]. This negative feedback mechanism effectively keeps the activity of NF-κB in check. These findings not only provided insights about miRNA-mediated inflammatory responses, but also of potential drug targets for fine-tuning the immune system.

Neurodegenerative diseases

Neurodegenerative diseases (ND) such as Parkinson's disease (PD) and Alzheimer's disease (AD) have placed substantial social-economic burdens on countries with aging populations. As the pathogeneses of NDs on molecular levels remain poorly understood, successful treatments are still unavailable. With increasing investments from governments and pharmaceutical companies, biomedical research on neurodegenerative diseases has become proprietary. Notably, recent progresses from studies elucidating miRNA functions in NDs have shed new light on disease pathogenesis and may lead to novel treatment strategies. For example, a systemic miRNA profiling in peripheral blood mononuclear cells from PD patients revealed miR-30b, miR-30c, and miR-26a to be associated with the susceptibility of the disease [91]. Deregulation of miR-133b expression may contribute to the pathogenesis of PD, as the miR-133b-Pitx3 feedback loop is essential for maintaining dopaminergic neurons in the brain [92]. In a *Drosophila model for PD,* pathogenic leucine-rich repeat kinase 2 (LRRK2) was shown to promote the expression of transcriptional factors E2F1 by down-regulating expression of let-7 and miR-184* [93]. Likewise, an analysis of miRNA and mRNA expression in brain cortex from AD and age-matched control subjects demonstrated strong correlations between the expression levels of miRNAs and predicted mRNA targets [94], implying functional relevance of microRNA-mediated regulations in AD pathogenesis. More specifically, the expression of

miR-29a, miR-29b-1 and miR-9 was significantly decreased in AD patients [95], resulting in abnormally high expression of their target BACE1, a protein playing an important role in AD pathogenesis [96]. These findings not only highlight the importance of miRNA research in understanding ND pathogenesis, but also provide a previously unrecognized venue for medical interventions.

miRNAs in disease diagnosis and therapy

While the combination of molecular and computational approaches have revealed the role for miRNAs in common human diseases, concurrent developments of miRNA biomarkers and miRNA drugs have made great strides towards improving public health.

The ultimate goal of biomarker identification is to develop better clinical tests that improve diagnosis or prognosis of diseases. In fact, miRNAs have been considered a top candidate for the next generation of biomarker as they possess a few advantages over other candidates such as proteins and metabolites [97]. First, miRNA biomarkers would more likely lead to early diagnosis due to their upstream positions in regulation cascades. Second, novel miRNA biomarkers would be more readily discovered by genomic tools such as oligonucleotide microarrays and deep sequencing which deliver higher throughput than mass spectrometry, the primary tool for protein and metabolite biomarker identification. Third, low abundant miRNA biomarkers can be amplified and then detected in a clinical setting by real-time quantitative PCR (qPCR), an approach used in FDA-approved clinical tests already; whereas, no equivalent approach is available in detecting low abundant proteins or metabolites. The adoption of the locked-nucleic acid (LNA) technology in miRNA probe design could improve the sensitivity and specificity of miRNA qPCR assays even further [98].

Non-invasive miRNA biomarkers are more sought after due to fewer complications associated with the specimen collection through the more prominent use of bodily fluids such as serum and plasma. In fact, circulating miRNA biomarkers have demonstrated early promises in diagnosis of prostate cancer [99], lung cancer [100,101], liver cancer [102] and breast cancer [103]. As circulating miRNAs are very stable in the blood [99,104], they could be well-preserved in archived serum or plasma specimens, a gold mine for miRNA biomarker development.

miRNA drug development is still in its infancy with the exception of SPC3649, a LNA-modified oligonucleotide developed by Santaris Pharma A/S to repress the expression of miR-122, in treating chronic HCV infection. This miRNA drug demonstrated impressive repression efficacy on miR-122 in mice [105] and in African green monkeys [106], as well as anti-viral efficacy in chimpanzees chronically infected by HCV [74]. Compared to a combined administration of pegylated interferon-α and ribavirin, the standard treatment for HCV infection, SPC3649 demonstrated better safety profiles in chimpanzees [74] and desired tolerance in healthy volunteers. Importantly, the SPC3649 treated patients rarely experienced viral-relapse, whereas viral-relapse is common in patients treated with pegylated interferon-α. Interestingly, the expression of interferon-regulated genes decreased in parallel with HCV titers during the SPC3649 treatment. This indicates the effectiveness of SPC3649 on patients infected with viral strains resistant to the interferon-α treatment.

Future directions

In spite of the early success of SPC3649, few miRNA drugs have entered clinical phases due to two major challenges. First, currently available target prediction softwares have high false-positive rates, making it difficult to identify a bona fide miRNA target by *in silico* prediction alone [107]. To better predict a miRNA drug target before entering costly animal and clinical studies, researchers should take the advantage of combining molecular biology and

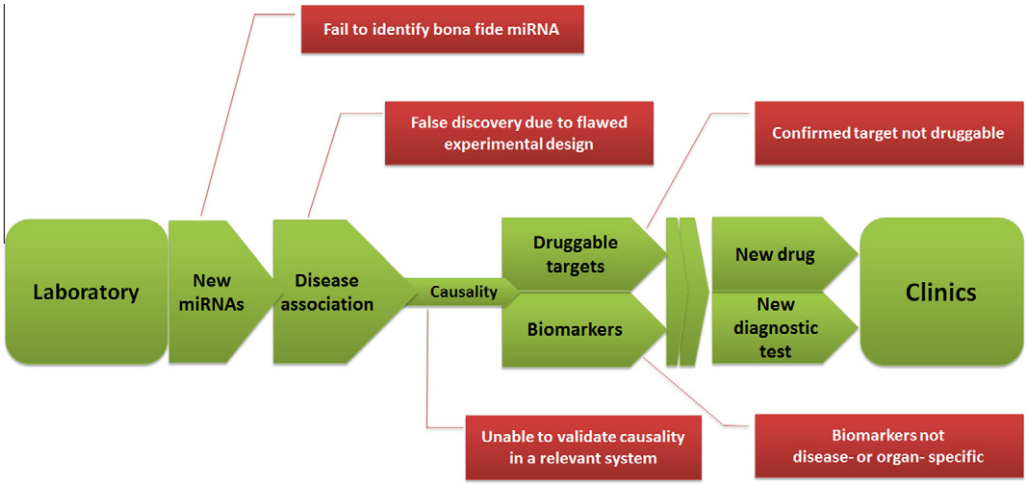

Figure 1 The road from laboratory to clinic: the promises and challenges of miRNA research
The hopscotch course in green is a layout of an ideal path of miRNA research evolved from basic research to clinical practice. Red boxes indicate major challenges at different steps.

bioinformatic approaches in target prediction and validation. Recent advancement of molecular biology techniques, such as RISC immune-precipitation and Argonaute-protein crosslinking immune-precipitation, provide valuable tools allowing target enrichment before bioinformatic predications [108,109]. These techniques should be fully integrated into the studies for target identification. Second, the effective dose of a miRNA drug may induce unsafe off-target effects. A cocktail regimen of miRNAs collaboratively repressing the same target at low doses could be a potential solution. This strategy requires not only extensive bioinformatic efforts in drug designs, but also high-throughput genomic screening to validate the drug effects.

Concluding remarks

Without a doubt, the importance of miRNA is gaining appreciation. However, even with its already demonstrated promise, miRNA diagnosis or therapy may be many years away from entering the clinic as complex challenges remain (**Figure 1**). It should be noted that any major leap forward in miRNA research over the past decade was the result of multidisciplinary collaborations of researchers with extensive expertise in molecular biology techniques, high-throughput genomics, and bioinformatics. These productive collaborations should be expended even further. With clinicians joining the club, miRNA research will be given a fresh perspective that may lead to steady progress in development of clinical applications.

Competing interests

The authors declare no conflict of interests.

Acknowledgements

We thank Bryan Maliken for editing assistance. This work is supported by the grants from NIDDK (Grant No. 3R01DK056924-08S1 and 5K24DK002957) and NHLBI (Grant No. 1R21HL112678).

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