Published in final edited form as:

Transl Res. 2011 April ; 157(4): 157–162. doi:10.1016/j.trsl.2011.02.001.

MICRORNAs: miRRORS OF HEALTH AND DISEASE

Monty Montano

Boston, MA

Abstract

The review articles in this issue provide an improved appreciation for miRNA as an essential feature of lineage commitment and regulatory guidance during tissue development, that when absent or hampered, often lead to disease states. In the coming years, there is much to be learned about adaptive (and maladaptive) states by examining how expression of miRNAs is influenced by the genetic architecture of miR genes, clusters and mirtrons, as well as miRNA polymorphism and polymorphism in their mRNA targets. We were also introduced to several modes of miRNA regulation (negative feedback, positive feedback, cross-regulatory) that monitor, modulate or resolve signaling pathways in a variety of biological processes that include sepsis response, fibrosis, acute exercise and steroid biology. Perhaps the homeostasis or micromanagement of these miRNA regulatory systems, when perturbed arrive at new stable networked interactions that have an undesired effect of promoting or antagonizing disease severity and cancer progression. Clearly, a better understanding of these miRNA regulatory networks, as well as improved therapeutic tools for guiding miRNA expression and their targets towards desired outcomes will be the subject of many advances in miRNA biology over the coming years.

INTRODUCTION

In 1993 the laboratories of Ambros and Ruvkun made the remarkable observation that the *lin-4* gene coded for short RNAs that governed development of the nematode *Caenorhabditis elegans* through translational repression of *lin-14* (5,6). Some years later, the Ruvkun lab identified a second example of a small regulatory RNA, *let-7*, that had orthologues in other species, suggesting that a novel regulatory mechanism was potentially conserved in evolution. When a large number of small RNAs were revealed in 2001, the microRNA field was effectively launched (8–10). Since 2001, there has been a profound re-interpretation of regulatory biology, as well as, the recognition of an entirely new class of therapeutic tools based on the reach of microRNA into virtually every field of molecular medicine.

In this special issue of *Translational Research*, several articles review the complex role for microRNAs (miRNAs) in the pathobiology of multiple organ systems that include the lung, heart, kidney, liver, skeletal muscle, and immune system. The reviews, as an ensemble, touch upon several themes in our current understanding of microRNAs, and it is these themes that are in the subject of this commentary. An essential role for the control of post-transcriptional gene expression is underscored by the affects of knocking out key regulators of miRNA biogenesis. The adaptive capacity of the miRNA machinery to modify the protein

Address: Boston University Medical Campus, Department of Medicine, 650 Albany Street, EBRC 646, Boston MA 02118, mmontano@bu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

profile of the transcriptome, in response to environmental cues is evident in the genetic organization and variability of their loci, as well as in the elaborate feedback systems that are utilized, or coopted in diseases such as cancer, fibrosis, sepsis and autoimmune disease. Current efforts to leverage knowledge of this regulatory system to diagnose, track and attenuate disease progression, represent a major new research opportunity and challenge in this rapidly growing area of translational medicine.

1. Knockout of miRNA biogenesis: small RNAs with big effects

As our understanding of miRNA biogenesis and downstream regulatory activities unfold, so too does our appreciation for the extent and scope of influence these small RNAs have on multiple biological processes across human health and disease. Multiple reviews in this issue discuss the deleterious effects of knocking down miRNA biogenesis, generally by focusing on Dicer endonuclease loss of function, either globally or tissue specifically. Dicer knockdown, in effect, disables the processing of pre-miRNA, an obligatory first step in the RNA interference pathway and formation of the RNA inducing silencing complex (RISC). Without RISC formation, argonaute mediated degradation of target mRNA is lost. For an in depth review of miRNA structure and function, see 1.

In this issue, Dmitrovksy and colleagues² discuss experimental knockouts of Dicer and how the loss of this miRNA biogenesis step can impair global miRNA processing and enhance tumor susceptibility in murine models of cancer. Dorn and colleagues³ note that when Dicer is selectively ablated in cardiac myocytes there is a resulting cardiomyopathy. Becker and colleagues⁴ discuss how conditional knockdown of Dicer impairs normal kidney development. And further, when specific Dicer knockout in kidney podocytes is achieved, this leads to proteinuria and glomeruli abnormalities in mice, further emphasizing the normal and dysregulatory potential in microRNA processing. Regazzi and colleagues⁵ discuss the observed abnormal lipid buildup and eventual steatosis resulting in hepatocytes obtained from Dicer null mice. Similarly, Kerr and colleagues⁶ discuss how liver specific Dicer deletion at three weeks lead to progressive steatosis. Although the authors caution that this phenotype has not been universally observed. While unlikely to change the overall conclusion that miRNAs clearly play a significant role in regulating multiple pathways, the subtleties of distinct phenotypes in these conditional knockdowns may hinge upon the build up of precursor miRNAs that may activate other RNA surveillance pathways, such as RNA editing, which would then introduce an additional variable^{7, 8}. Comparative transcriptome profiling with high throughput RNA sequencing will provide more insight into the global phenotype of these knockdowns.

2. Genetic organization and variation in miRNAs

Canonical miRNAs are generated from protein-coding transcriptional units; whereas, other miRNAs (i.e., non-canonical miRs) are encoded in non-coding transcriptional units. In both cases, the miRNAs can be located either within intronic or exonic regions. As Gerald Dorn 3 points out in his review, miRs tend to be organized in a related cluster and also tend to target multiple mRNA transcripts within common cellular response pathways (e.g., proliferation, apoptosis). This organizational thematic provides miR clusters with a capacity for coordinate regulation of multiple steps within a pathway, providing an opportunity for complex and adaptive regulatory control. A noteworthy mechanistic distinction in canonical vs. non-canonical miRNAs is that canonical intronic miRNAs are Drosha-dependent (i.e., requiring Drosha endonucleolytic cleavage to be processed to pre-miRNA) and processed co-transcriptionally with protein-coding transcripts in the nucleus. The pre-miRNA then enters the miRNA pathway, whereas the rest of the transcript undergoes pre-mRNA splicing to produce mature mRNA slated for protein synthesis. Non-canonical intronic small RNAs(also called mirtrons) can derive from small introns that resemble pre-miRNAs, and

bypass the Drosha-processing step. Ahmed and colleagues 9 discuss interesting features of these non-canonical miRNAs. Gerald Dorn³ discusses an interesting class of miRs termed myomiRs – so called because they are coded within myosin heavy chain MYH genes. myomiRs are transcribed in the same precursor mRNA as parent MYH gene. Of special note is the myomiR-499, which despite the absence of a parent mRNA, is one of most highly expressed miRNAs in heart tissue. In an apparently novel evolutionary phenomenon, alternative splicing in the heart uncouples production of mature miR-499 from expression of parent MYH7b mRNA, meaning that the mRNA has evolved into a non-functioning host for its incorporated intronic miR (i.e., mir-499).

Comparative studies evaluating the organizational structure of the mammalian genome have identified a wealth of chromosomal insertion-deletions (indels), copy number variants (CNV) and single nucleotide polymorphisms (SNPs) that, depending on the environmental context, contribute to the genetic variation that underlays phenotypic diversity. This diversity is evident in nearly every aspect of human health and disease that has been investigated. There is now a growing recognition that variation in microRNAs and their target genes also contribute to this phenotypic complexity. Croce and colleagues 10 point out the observation that several solid and hematologic malignancies can be linked to miRNAs located at amplified, deleted or translocated chromosomal regions in the mammalian genome. Additionally, Zhang and colleagues 11 point out the notion that variation in gene expression and regulation is likely influenced by genetic variants in cis- and trans- acting SNPs (also known as eQTLs, expression quantitative trait loci). The authors also discuss an interesting class of miRNA binding site polymorphisms (miRSNPs) within functional genes. For example, miR-24 appears to be deregulated in human colorectal tumor through a target site polymorphism in the dihydrofolatereductase gene (DHFR). In another example not cited in these reviews, a polymorphism within the myostatin gene creates a target site for mir-1 and mir-206, which are highly expressed in skeletal muscle. This causes translational inhibition of the myostatin gene and hence contributes to the observed muscular hypertrophy 12. Zhou et al¹¹ make the important observation that given the significant differences in gene expression variation across human populations, analysis of miRNAs in regulating population differences in gene expression is likely to provide substantial insights in to health disparities.

It is also worth noting that comparative genomics studies indicate that the target mRNA sequences for miRNAs: untranslated regions (UTRs) display sequence diversity suggestive of genetic drift. This may suggest that over the course of evolution there is opportunity for a continuous sampling of co-expressed miRNAs and cognate mRNAs with these UTR variants. As recently discussed by David Bartel¹³, depending on whether the dampening of protein output is beneficial, inconsequential, or harmful, the UTR sites may be selectively conserved, neutral, or avoided during miRNA: mRNA coevolution.

3. miRNA regulatory complexity: feed-back loops and environmental sensing

Perhaps one of the most salient and unexpected features of miRNA is the role for miRNA in amplifying or tempering cell signaling. This modulatory capacity provides an adaptive tool that allows the initiation of cellular signaling to be calibrated to accommodate cues from the microenvironment, as well as to potentially buffer signaling. This buffering function has been proposed to function as a network stabilizing effect in the context of interlocking feedback loops and genetic variation 14. Some discussed examples of dysregulation in feedback properties of miRNAs are discussed in this issue. Kaminski¹⁵ and colleagues discuss a role for miR-21 in the lung in a feed-forward loop that promotes TGF- β amplification and fibrosis through de-repression of a miR-21 target mRNA, the inhibitory Smad 7. Interestingly, TGF- β both inhibits miRlet-7 and upregulates miR-21. These two microRNAs appear to be functionally opposed in lung tissue from human subjects with

idiopathic pulmonary fibrosis (IPF) lungs (let-7 acts as a negative regulator and miR-21 as a positive regulator) that in effect balance the fibrotic phenotype.

Zhou 11 and Dai⁹ both discuss a pioneering study that link miRs with innate response regulation based on the observation that miR-146 and miR-155 are rapidly upregulated during endotoxin/LPS stimulation of human monocytic cells. In this context, miR-146a appears to function as a negative feedback regulator by targeting IL-1 receptor associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6). A reciprocal negative feedback is achieved through MAP kinase phosphatase -1 (MKP-1) mediated suppression of miR-155, which in turn targets suppressor of cytokine signaling-1, SOCS-1). Thus, MKP-1 appears to function as a de-repressor of miR-155 mediated suppression to modulate LPS response in mouse macrophages.

Guay et al⁵ also describe a feedback loop in muscle between miR-1 and insulin growth factor -1 (IGF-1), based on the use of a skeletal muscle precursor cell line (C2C12) and in cardiac muscle tissue. In the C2C12 cell line, miR-1 targets IGF-1 and the IGF-1 receptor. Interestingly, IGF-1 also reciprocally regulates miR-1 via the transcription factor Foxo3a, apparently through an enhancer binding element within the miR-1 promoter. Guay and colleagues⁵ also discuss interesting data suggesting that changes in miRNA levels can be detected in response to endurance or resistance exercise. In a related discussion on anabolic modulators, by Dai et al⁹, they discuss estrogen and androgen modulation of miRNA levels. Notably, estrogens appear to induce miR-451, -486, -223, -148a, -18a, -708; as well as suppress miR-146a, -125a/b, -143, -145, let-7e, -126 and miR-181a. Dai et al⁹ also discuss work describing a role for androgens in the regulation of miRNA in non-lymphoid organs including prostate, muscle and liver, as well as the mutual interaction between miRNA and androgen receptor signaling in prostate cancer.^{168–171}

4. Organ diseases and miRNAs

A potentially important and exploitable distinction between disease and healthy states in tissue homeostasis may reside in the regulatory networks that distinguish these states. There is a growing recognition that miRNA networks are often associated with tissue dysfunction and are likely to be a key source of altered gene expression that underlays and distinguishes healthy tissue from dysregulated tissue. A better understanding of the key perturbations that lead to these alternative states will likely inform therapeutics. Early work by Spira and colleagues¹⁶ discuss multiple profiling studies of bronchial airway epithelia, wherein in one example, 28 miRNAs were downregulated in smokers that could be correlated with mRNA target expression in vivo. Becker and colleagues⁴ discuss an observed differential expression of microRNAs, notably miR-155, in different kidney compartments (i.e., kidney cortex versus kidney medulla regions). Also noted is that in individuals with trisomy 21, fibroblasts display higher levels of miR-155.

Also interesting is the expression of miR-192 in diabetic nephropathy, with a suggestive role in mediating TGF- β induced collagen expression. In that study, deletion of the inhibitory Smad7 promoted miR-192 expression, in a model for obstructive kidney disease. Guay et al⁵ also note that mRNA profiling can be altered in the diabetic state, based on the observation that there is an apparent discordance between proteomic profiles and their respective mRNA levels, suggesting a potential role for microRNAs. Interestingly, insulin secreting cells exposed to pro-inflammatory cytokines display elevated miR-21 levels and miR-146 levels are increased in a murine model for type-2 diabetes (db/db mice). Additional studies on insulin effects using a glucose clamp also indicate that in skeletal muscle, insulin was associated with a reduction in 39 miRNAs including miR-1, miR-206 and miR-133a (all canonical muscle regulatory miRs), in a process that was arguably mediated by transcription

factors sterol regulatory element -binding protein -1c (SREBP-1c) and myocyte enhancer factor 2C (MEF2C).

Kerr et al 6 note that in liver miR-122 is one of most abundant miRNAs and that knockdown of miR-122 decreased hepatic lipogenesis, resulting in a protection from high fat diet induced hepatic steatosis.⁶ and also note that approximately 46 miRNAs were either upregulated or downregulated in liver disease (e.g., non-alcohol fatty liver disease, NAFLD).

Dai and colleagues⁹ summarize evidence for the critical role of miRNA in immune system homeostasis. For example, mir-155 was upregulated in T and B cells upon activation and required for the maintenance of lymphocyte homeostasis, notably production of Th17 promoting cytokines. Mice with an ablation of miR-146a specifically in regulatory T cells (Tregs) display elevated levels of the T helper -1 (Th1) cytokine interferon-gamma (IFN- γ) a breakdown of immune tolerance with an increased risk for autoimmune disease. Several additional studies describe a dysregulated miRNA profile in blood cells from individuals with lupus, an autoimmune disease characterized by production of auto antibodies). Upregulation of miR-155 in lupus B and T cells may lead to the characteristic abnormal B cell responses in germinal centers. Dysregulated expression of the microRNAs miR146a and miR-155 in synovial fibroblasts is also implicated in rheumatoid arthritis (RA). Notably, although miR-146a was upregulated in active RA disease, the target genes, IRAK-1 and TRAF6 had no apparent change in their levels when compared to healthy controls. This result may indicate that miRs may lose their capacity to function as negative regulators of inflammation in the context of RA.

5. Cancer and microRNAs

Perhaps one of the most critical developments in the pathobiology of microRNAs has been the recognition that miRNAs modulate the transcriptome to either reflect a cancerous state, or promote or attenuate cancer risk. Work by Spira and colleagues¹⁶ have noted that reduced expression of miRNAs has often been associated with dedifferentiated tumors in lung epithelial cells and Pandit et al¹⁵ also discuss downregulation of epithelial markers as suggestive of an epithelial-mesenchymal transition (EMT), a key developmental program that is often activated during cancer invasion and metastasis, that is in this case diagnostic of lung cancer. Nana-Sinkham et al¹⁰ make the observation that global repression of miRNA expression appears to be a common event in cancers. Of note they describe how reduced lung tumor expression of Dicer, as well as reduced Dicer occurs in in advanced adenocarcinoma.

Kerr et al 6 discuss how miRNAs may promote or attenuate the oncogenic phenotype by either decreasing expression of tumour suppressor genes (oncomiRs) or alternatively by targeting oncogenic mRNA for silencing (tumour suppressor miRNAs). Nana-Sinkham et al 10 discuss the role of Let-7 as a tumor suppressor, which is perhaps the most studied miRNA in cancer. Let-7 over-expression has been shown to inhibit tumor development and growth. Nana-Sinkham¹⁰ and Pandit¹⁵ note that miRs (e.g., miR-17-92, miR-21) may function as oncomirs and are linked to regulators of cell cycle to promote proliferation, e.g., CDKN1A(p21), E2F family and PTEN, respectively. Nana-Sinkham et al¹⁰ describe an interesting correlation between low let-7a-2 and high miR-155 in association with poor survival in adenocarcinoma of the lung. In studies discussed, comparative miRNA expression in adenocarcinoma and squamous cell carcinoma revealed a signature of 34 miRNA in male smokers and 5 miRs that predicted survival in a cohort of 107 smokers with early stage squamous cell carcinoma.

Notably, Liu et al (Liu et al., 2011) highlight an additional complexity; namely, that the cell and tissue context of miR expression can be critical to understanding the overall pathobiology of cancer. For example, miR-31 appears to be pro-oncogenic in lung cancer and alternatively protective in breast cancer. They also note that elevated miR-31 is observed in lung cancer cell lines, and that engineered knockdown reduces cellular growth and colony formation.

6. MiRNA therapeutics and target prediction: successes and challenges

The prospect of leveraging the growing knowledge of miRs and their respective targets to achieve therapeutic outcomes has prompted an explosion of research to identify miR modulators with applications for a broad range of disease conditions. Regazzi and colleagues⁵ provide an overview of the nomenclature of chemically modified miRs that include locked nucleic acid (LNA), anti-miRs, antagomiRs, morpholinos and miR sponges – with each there is the promise of efficient inhibition of miRNA function and evidence for effective activity *in vivo*. A particularly intriguing example of their use to date involves the use of miRNA sponges as miRNA decoys against specific miRs in Type 1 and Type 2 diabetes. The use of an *in vivo* antagomir antisense oligonucleotide for miR-122 (one of the most abundant miRs in liver that has been associated with cholesterol regulation) resulted in almost complete depletion of miR-122 in hepatocytes and a decrease in hepatic fatty acids, cholesterol synthesis and plasma cholesterol levels. Additional successes in the use of miR based therapeutics are described by Nana-Sinkham et al¹⁰; wherein they review one study of the systemic delivery of a oligonucleotide-LNA of miR-122 in a non-human primate model for chronic HCV. In those experiments there is early success, with evidence for a potent reduction in HCV burden. Nevertheless, as Gerald Dorn points out³, an important caveat and challenge in the design of miR therapeutics will be estimating the likelihood for “off-target” binding or quenching (in the case of miR sponges) and designing safeguards against undesirable outcomes.

With the increasing sophistication of bioinformatics tools for probing genome wide sequence features, and the wide accessibility of reference genomes for several organisms, most notably human, mouse and nematode, there has been an explosion of web accessible algorithms designed to predict genomic interactions between miRNA and putative mRNA. This has been coupled with substantial progress in the *in vitro* validation of sequence recognition criteria for miRNA:mRNA target interaction. Balancing many successes, as Zhang¹¹ and colleagues point out, miR binding predictions based on the use of the most popular computational tools (i.e., miRanda, Target Scan, PicTar) have not generally been correlated to each other and their predictions for interaction are often not supported by experimental evidence. Clearly refinements, based on more empirical evidence and theoretical considerations will be needed to expand the utility of these tools to interrogate complex rules for genomic interaction.

One level of improved sophistication, as Gerald Dorn notes³, will be the implementation of additional limitations in these bioinformatics tools to incorporate critical information obtained from empirical data for miR and mRNA expression differences. In the context of different states, e.g., healthy and disease tissue, this will likely influence global target recognition and overall expression profiles. One approach to this concern of contextual expression of miRNA is discussed by Dorn and colleagues³ who have championed the use of high throughput sequencing procedures coupled to immunoprecipitation of miRNA:mRNA complexes bound within the RISC complex. In a technique termed “RISC-Seq”, RISC complexes are immunoprecipitated (e.g., using anti-Argonaute 2 antibodies), followed by RNA sequencing. This approach allows for precise mapping of target sequences in the context of over-expressed miRNAs and will likely be instrumental in profiling tissue and disease specific miRNA expression.

7. Circulating miRNA as biomarkers

There is substantial interest in identifying circulating biomarkers that are useful in classifying disease severity and in gauging therapeutic response to candidate drug interventions. With the surprising observation that microRNAs are detectable in serum and are relatively stable - due to protection in extracellular vesicular structures called exosomes, there has been a substantial new growth and enthusiasm for this line of research. In this issue, many reviews discuss the remarkable utility of miRNAs as circulating biomarkers for a host of disease phenotypes. For example, in non-small cell lung cancer (NSCLC), Liu and colleagues (Liu et al., 2011) describe studies identifying a four miRNA (-486, -30d, -1, -499) serum signature. Kratzke and colleagues¹⁷ also discuss many of the caveats to progress in biomarker identification for lung cancer. Foremost being the need for RT-PCR as a validation for miR profiling and novel solutions to the difficulty of normalizing biomarker levels in serum. Pandit¹⁵ and colleagues also discuss progress in identifying biomarkers for sepsis (e.g., miR-150 in plasma). Zhang¹¹ and colleagues describe studies linking serum miR-146a and miR-223 as potential biomarkers for sepsis and that miR-150 can be correlated with aggressiveness of sepsis, and as such, may be a prognostic marker in patients with sepsis. Guay^{et al}⁵ summarize evidence for serum miRNAs in diabetes. In one study, serum miRNA profiles from diabetic and healthy subjects identified 65 common and 42 differently expressed miRNAs. Studies using pooled sera have identified 13 miRNAs in diabetic patients compared with healthy subjects, further highlighting the promise of miRs as serum or plasma biomarkers. Finally, in Gerald Dorn's review³ he describes evidence for microRNAs as circulating biomarkers in cardiac disease and summarizes work by Corsten et al who observed a striking 1000-fold increase in miRs-208b and -499 after myocardial infarction. Collectively, in the coming years, it will be interesting to see how sensitive and stable these miR circulating biomarkers are and to what extent these peripheral markers reflect organ health.

References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004; 116:281–97. [PubMed: 14744438]
2. Liu X, Sempere L, Guo Y, et al. Involvement of MicroRNAs in lung cancer biology and therapy. *Translational Research*. 2011;157. [PubMed: 21420026]
3. Dorn G. MicroRNAs in cardiac disease. *Translational Research*. 2011;157. [PubMed: 21420026]
4. Akkina S, Becker B. MicroRNAs in kidney function and disease. *Translational Research*. 2011;157. [PubMed: 21420026]
5. Guay C, Roggli E, Nesca V, Jacovetti C, Regazzi R. Diabetes mellitus, a microRNA-related disease? *Translational Research*. 2011;157. [PubMed: 21420026]
6. Kerr T, Korenblat K, Davidson N. MicroRNAs and liver disease. *Translational Research*. 2011;157. [PubMed: 21420026]
7. Long K, Montano M. RNA surveillance-an emerging role for RNA regulatory networks in aging. *Ageing Res Rev*. 2010
8. Nishikura K. Functions and regulation of RNA editing by ADAR deaminases. *Annu Rev Biochem*. 2010; 79:321–49. [PubMed: 20192758]
9. Dai R, Ahmed A. microRNA, a new paradigm for understanding immunoregulation, inflammation and autoimmune diseases. *Translational Research*. 2011;157. [PubMed: 21420026]
10. Nana-Sinkham P, Croce C. microRNA as therapeutic targets in cancer. *Translational Research*. 2011;157. [PubMed: 21420026]
11. Zhou T, Garcia J, Zhang W. Emerging Role of MicroRNAs in Inflammatory Lung Disease. *Translational Research*. 2011;157. [PubMed: 21420026]
12. Clop A, Marcq F, Takeda H, et al. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nat Genet*. 2006; 38:813–8. [PubMed: 16751773]

13. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136:215–33. [PubMed: 19167326]
14. Li X, Cassidy JJ, Reinke CA, Fischboeck S, Carthew RW. A microRNA imparts robustness against environmental fluctuation during development. *Cell*. 2009; 137:273–82. [PubMed: 19379693]
15. Pandit K, Milosevic J, Kaminski N. microRNAs in idiopathic pulmonary fibrosis. *Translational Research*. 2011;157. [PubMed: 21420026]
16. Schembri F, Sridhar S, Perdomo C, et al. MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. *Proc Natl Acad Sci U S A*. 2009; 106:2319–24. [PubMed: 19168627]
17. Yendamuri S, Kratzke R. MicroRNA biomarkers in lung cancer: miR'acle or quag'miR'e? *Translational Research*. 2011;157. [PubMed: 21420026]