MicroRNA:
An Insight to miRNA-based Microarrays, Diagnostics and Therapeutics

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New to Insight Pharma Reports is: MicroRNA: An Insight to miRNA-Based Microarrays, Diagnostics and Therapeutics. This report covers the specifics of microRNA (miRNA) as it is a relatively new space in heavy pursuit of research due to its versatility and stability, properties that are unique to miRNAs compared to other RNA components. miRNA’s non-coding nature and ability to affect genetic expression make it a novel candidate for use as a biomarker for a variety of diseases. This prompted the pursuit of miRNA-based microarrays and diagnostics for the advancement in therapeutic development. Furthermore there are a number of academic laboratories not only validating miRNAs, but also identifying them in a number of diseases and examining their molecular actions in the presence of other molecules. This report is broken up into several parts, highlighting miRNA in several areas of research.

After covering background information, the second and third parts of this report expand upon several microarrays and diagnostics for specific analysis and therapeutic development. Several companies featured in this section include:

• Affymetrix  
• Exiqon  
• Diamir  
• Rosetta Genomics  
• Eisai

Chapters within these sections feature company backgrounds and highlight proprietary technologies and platforms. Additionally, several advantages and limitations are also discussed. More significantly, these chapters are accompanied by interviews with company representatives, exclusively conducted for this report.

The fourth part of this report explores the academic, research and community perspectives. Although not developing diagnostics per se, academia is diligently investigating several aspects of miRNAs including their molecular properties for use as biomarkers for neurodegenerative diseases, diabetes, and even cancer. Universities and research institutes included in this area are:

• Stony Brook University School of Medicine  
• Grand Valley State University  
• Van Andel Research Institute

Also included in these sections are interviews with experts in leading research from these universities. From a community perspective, Dr. Argyropoulos, a Medical Affairs Consultant, provides insight to the rapidly growing space and rising opportunities.

Finally, the report concludes with an in depth analysis of survey results. With 152 researchers, these results provide an overall perspective of the research community, capturing a number of miRNA applications, challenges afloat in the industry, and miRNA mechanisms of study.
Executive Summary

MicroRNA: An Insight to miRNA-Based Microarrays, Diagnostics and Therapeutics is a new report to Insight Pharma Reports. microRNA (miRNA) is a relatively new space in heavy pursuit of research because of its versatility and stability, properties that are unique to miRNAs compared to other RNA components. Characterized by its non-coding nature, yet its ability to affect genetic expression, these features make miRNA a novel candidate for use as biomarkers for a variety of diseases. This prompted several companies to pursue miRNA-based microarrays and diagnostics for the advancement in therapeutic development. Furthermore, there are a number of academic laboratories not only validating miRNAs, but also identifying them in a number of diseases and examining their molecular actions in the presence of other molecules.

This report is broken up into five parts. After covering brief background information, the second and third parts of this report expand upon microarray analysis, oligonucleotide synthesis, and diagnostics in development. Several companies featured in these sections include: Affymetrix, Exiqon, Diamir, Rosetta Genomics, and Eisai. Chapters within these sections feature company backgrounds and highlight proprietary technologies and platforms. Additionally, several advantages and limitations are also discussed. Even more significant, these chapters are accompanied by interviews with company representatives exclusively conducted for this report.

The fourth part of this report explores the academic research and community perspectives. Although not developing diagnostics per se, academia is diligently investigating several aspects of miRNAs including their use as biomarkers for cancer, neurodegenerative diseases, and nephropathy. Universities interviewed in this space include Stony Brook University School of Medicine, Grand Valley State University, and Van Andel Research Institute. Furthermore, Dr. Argyropoulos, a Medical Affairs Consultant, provides insight to this rapidly growing field and highlights advances in his research. Also included in these sections are interviews with leading experts from these universities and institutions.

Finally, the report concludes with an in depth analysis of survey results. With 152 researchers, these results provide an overall perspective of the research community, capturing an accurate representation of research demographics, challenges afloat in the industry, and market outlook of miRNA.
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About Insight Pharma Reports

CHI's Insight Pharma Reports are written by experts who collaborate with CHI to provide a series of reports that evaluate the salient trends in pharmaceutical technology, business, and therapy markets.

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CHAPTER 1

What is miRNA?

MicroRNAs (miRNAs) are a very popular area of interest. They are short sequences (roughly 21-26 nucleotides) of RNA that are produced via specialized RNA transcripts coded in the genome. Since their discovery in 1993 by Victor Ambros, Rosalind Lee, and Rhonda Feinbaum, miRNAs have been pursued in several areas including diagnostics and therapeutics. In order to understand the role of miRNA in genetics, this section will briefly discuss the process of transcription and gene expression.

Transcription

After DNA synthesis, DNA is transcribed into small segments of RNA, known as messenger RNA (mRNA), through the combined work of many proteins. These segments are further analyzed by even more proteins, which facilitate an action called splicing. This process occurs to differentiate introns from exons. Introns, or “junk DNA,” are DNA not used for translation purposes. Exons contain segments of genetic information which may be eventually translated into proteins. However, there are a lot of mechanisms which affect genetic expression including histone acetyltransferases, methylation, RNA stability and many others. One of the most notable players in genetic expression is microRNA.

Gene Expression

After DNA is transcribed into mRNA it is translated into proteins, which express various phenotypic and genotypic characteristics. microRNA plays a significant role in genetic expression as it binds to the 3’-UTR (untranslated region) of their target mRNAs. This destabilizes the mRNA and represses its protein production, inducing translational silencing. A similar RNA subtype is siRNA (small interfering RNAs). However, instead of suppressing genetic expression, this type of RNA actually leads mRNA down a path of destruction; any information is degraded and never has the opportunity to be translated. Based on findings, researchers have discovered that the key difference between suppressing genetic expression and destroying any translatable material revolves around the binding of the molecule.

“In siRNAs, a perfect match with the target mRNA marks the duplex for destruction by endonucleases. But most miRNAs do not match the target sequence exactly, and are unable to distinguish tiny variations in the recognition site.”

-Steven Buckingham
miRNA Creation and Application

Engineering Oligonucleotides

miRNAs offer a novel approach to diagnostic and therapeutic applications. However, in order to achieve these robust applications, miRNAs first need to be detected and analyzed. Due to poor detection methods, several companies have been taking different approaches to improve miRNA sensitivity and specificity. One of the ways to solve this issue is by synthesizing oligonucleotides with preemptively optimized properties.

Affymetrix is one company working on perfecting microarray technologies. They are working on enhancing the detection of miRNAs across a number of research areas by using a photolithographic process to synthesize oligonucleotides. Another company highlighted in this section is Exiqon, which features locked nucleic acid as its primary method for enhancing miRNA detection and therapeutic application. This section will discuss several methods to synthesize oligonucleotides.

Photolithographic Synthesis

Photolithographic synthesis seems to be most commonly utilized by Affymetrix for their GeneChip technology. This application is a light-directed oligonucleotide synthesis; more specifically the methodology employs MeNPOC photo-activatable nucleoside monomers. With an ability to print individual 10µm² probe features at a density of 10⁶ probes/cm², this method facilitates the "widespread use in the hybridization-based detection and analysis of mutations and polymorphisms." Ultraviolet (UV) masking, in combination with light-directed chemical synthesis, selectively synthesizes probes located directly on the surface of the array. These probes contain a protecting group on their free end that is activated once in contact with the UV light. A photolithographic mask directs the UV light in such a way that exposure to specific nucleotides can be controlled. It works by deprotecting, and therefore activating, a series of hydroxyl groups. This effect initiates coupling with incoming protected nucleotides that attach to the activated sites. Although each probe on the chip requires four masks per round of synthesis, this method features increased sensitivity and specificity that enable discrimination of single mismatched base pairs.
their Philadelphia-based CAP accredited, CLIA certified lab: “The fundamental basis for all of our current microRNA-based diagnostic testing services is the measurement of microRNA expression profiles that are specific to discrete histological subtypes.” Berlin explains that Rosetta Genomics uses a combination of microarray technology and qRT-PCR to detect microRNA gene expression. The company developed several tests that use “advanced bioinformatics to generate algorithms to convert microRNA expression into final results.” Furthermore, these tests can also distinguish between cell types “by measuring the unique microRNA expression profiles found specifically in different cell types or histological subtypes.” The testing services that Rosetta Genomics offers include: Rosetta Cancer Origin Test™, Rosetta Lung Cancer Test™, Rosetta Kidney Cancer Test™, and Rosetta Mesothelioma Test™. Descriptions and specifications of each test are outlined in Table 6.1.

Berlin describes that these tests can be used in tandem with other diagnostics, as well as with each other, on the molecular level. For the Rosetta Cancer Origin Test™ and the Rosetta Kidney Cancer Test™, Berlin explains that target-specific oligonucleotides are spotted onto custom microarray slides. From there, extracted RNA (labeled with fluorescent dye) is incubated with the slides. This allows the target microRNA to hybridize with the oligonucleotide probes. “The expression of the microRNAs,” Berlin continues, “is then measured by an analyzer and signals are then input into the individual assay algorithms.”

Regarding the Rosetta Lung Cancer Test™ and the Rosetta Mesothelioma Test™, instead of using specific oligonucleotides on microarray slides, extracted RNA is amplified and quantified using qRT-PCR: “This allows target microRNAs to be amplified and analyzed utilizing fluorescent probes.” Because of the platform’s high sensitivity and specificity, it allows for “quantification of minute amounts of microRNAs in the tissue.”

Speaking to validation, Berlin explains the process conducted via retrospective, blind studies: “All test algorithms were “trained” in studies using known samples, and then validated on real samples where a diagnosis had already been made using “gold-standard” work-up.” These tests improve upon currently available diagnostics because of their ability to “expand the range and accuracy of diagnosis,” particularly in the setting of small biopsy specimens. Even in diagnostic uncertainty, Berlin explains an objective answer is often possible and that “diagnoses not typically thought of” become apparent. With respect to future developments, Rosetta Genomics recently announced a pipeline of new products (Figure 6.1) “which contain both tissue-based cancer indications as well as body fluid-based non-cancer indications.”
**Figure 15.8. Assay Development: Technological Status**

What is the status of your most current technology?

- Launched/released: 16%
- Still in development: 58%
- Currently not working on any new technologies: 12%
- Non applicable: 14%

**Figure 15.10. Assay Development: Challenges Encountered**

What are the biggest challenges you've encountered in creating your assays?

- Integration with other products: 29%
- Market adoptability: 15%
- Validation: 48%
- Commercialization: 20%
- Financial limitations: 23%
- Other: 12%

**Figure 15.11. miRNA Therapeutic Market Outlook**

Where do you expect miRNA to be most effective as a therapeutic?

- Cancer: 46%
- Inflammation: 3%
- Pain management: 2%
- Virology: 6%
- Cardiology: 1%
- Fibrosis: 2%
- Metabolic diseases: 6%
- Biotechnology/nanotechnology: 7%
- Prenatal diagnostics: 2%
- Biomarkers: 16%
- Immunology: 4%
- Neurological/CNS: 2%
- Orphan diseases: 2%
- Other: 1%