The challenges of using blood-based miRNAs in the clinic

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The challenges of using blood-based miRNAs in the clinic


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MicroRNAs (miRNAs) are 22 nucleotide ribonucleic acids (RNA) involved in the post-transcriptional regulation of messenger RNAs (mRNA). mRNA regulation is a highly dynamic process, and miRNAs are profound effectors in this regulatory pathway. Cancers and other human diseases have elevated and decreased levels of specific miRNAs in circulation. The measurement of these miRNAs as biomarkers is a highly competitive area, given that early detection is critical for the overall survival of patients. To date, we still do not have any FDA approved miRNA assay. There is a wealth of data concerning miRNA expression in blood and tissue in a multitude of cancers and non-cancer diseases. Despite this, very few studies overlap in their identification of similar miRNA species, and we need to understand this lack of inconsistency.

There are numerous issues and major hurdles for the application of blood-based miRNAs, and we begin by suggesting a framework for standardizing miRNA testing in a clinical setting. A framework is necessary for eliminating errors between laboratories or testing sites. Moving forward, we then discuss the role of new technologies. We also evaluate the contribution of data analytics, and whether this approach will drive the next evolution of miRNA clinical research.

Standardization and automation

Standardization and automation are the cornerstones of pathology testing, permitting the robust detection of human diseases. Standardization coupled with automation eliminates variation between laboratories and errors associated with manual handling of specimens. Researchers in the field of miRNA biomarkers must adopt standardized guidelines for specimen collection, blood processing, and isolation techniques. Open source sharing of data without intellectual property constraints will also accelerate progress in finding consistency between studies. These challenges are not insurmountable, as pathology services and collection centers are already established in most countries. All that is required is an impetus for instilling our miRNA biomarker studies into this framework.

There is also an urgent necessity to formulate standard operating procedures for miRNA extraction from blood. All extraction protocols must be applied on a global scale, reducing the variation associated with different extraction methods. There are various approaches to isolating miRNAs, ranging from liquid-based guanidine isothiocyanate to silica columns. All have merits, but a consensus must be reached for ensuring consistency between different cohort studies.

Operating procedures must also extend to the detection of these miRNAs. Quantitative PCR (qPCR) data and next generation sequencing are the chief detection tools. Consolidating a universal miRNA extraction and detection pipeline will directly impact our ability to translate these miRNAs into the clinic. When these issues in collection, processing, and detection are resolved, there will be an enormous potential to use blood-based miRNAs as a screening program. Early screening and detection is one of the major factors in improving overall survival.

Research consortiums to accelerate clinical interest in miRNAs

In recent years, there has been a push for establishing large consortiums for miRNA research resulting in the generation of global surveys for miRNA expression patterns. These include programs such as the Circulating

Cell-Free Genome Atlas (CCGA), The Cancer Genome Atlas (TCGA), STRIVE Study, and FANTOM5. Several teams have curated the vast information and have developed websites for mining this data[1,2].

View the data: FANTOM5 miRNA data

View the data: A comprehensive, cell specific microRNA catalogue of human peripheral blood

Consortium-based research is also essential for aligning technical standardization across many different studies. Consortiums will also have funding support to run unbiased high-throughput screens in the discovery cohort, the most important stage. This improves the power and replicability of a study by detecting and quantifying large numbers of miRNAs in circulation. Multinational consortiums would agree on specific methodologies, reducing technical variation and accelerating the discovery of robust blood-based miRNAs. Considering the enormous scientific interest in miRNA biomarkers, research programs should be better designed, robust, powerful, and demonstrate clinical effectiveness.

miRNA detection methods

MicroRNA quantification is one of the major challenges in the clinic. Historically, the expression of blood-based miRNAs must be normalized to single or multiple (preferred) calibrator miRNAs or genes. Yet, in nearly all published studies, a different calibrator is selected for use. Inconsistent normalization profoundly influences the calculation of miRNA expression values, affecting the interpretation of results. Generally, as it is very difficult for clinical studies to find a consistent calibrator across different patient cohorts, researchers should present all their data, including raw Cq values and amplification efficiency. Two recent articles assist with study design by suggesting guidelines for selecting miRNAs with valid fluorescence values (i.e. above background noise), improving the accuracy and reliability of the published dataset[3,4].

Studies have now indicated that a calibrator gene may not be required at all[5,6]. We believe that alternative approaches such as base line fluorescence and normalization to the fluid volume will be the future methodologies for normalizing miRNA biomarker expression. Spike-in strategies are also implemented for normalizing miRNA expression, and we caution against this approach as it does not provide a true reflection of the biological content.

Accelerating miRNA biomarkers efforts with machine learning

Genetic data acquisition and analysis is improving exponentially. There is a wealth of information available from numerous databases around the world. FANTOM5 and TCGA are instances of repositories changing our approach to miRNA analysis. Most traditional wet labs lack the bioinformatic expertise in capitalizing on these new online sources. This remains one of the challenges for most laboratories requiring access to this information. Groups with bioinformatic expertise routinely employ machine learning to mine the data. Machine learning algorithms look for miRNA differences between thousands of cancer cases and healthy tissue in these

repositories. These custom algorithms are part of pipelines navigating and sweeping through these large swaths of data. Programmers assign a task to an algorithm, filtering through genetic markers and patterns, allowing the coded algorithm to consistently improve its learning ability in discovering novel patterns of expression. An example of this potential is the assistive AI tool from Mirocolus to build LoomBio, mirroring an open-sourced IBM Watson for health. From a manually selected dataset of 10,000 miRNAs, LoomBio was trained to provide an up-to-date miRNA snapshot.

Summary

There are many challenges facing researchers and companies invested in the search for clinically relevant miRNA biomarkers. However, miRNAs will have no meaningful clinical benefit until the research community produces consistent findings. As part of this community, we require a set of rigorous standard operating procedures with the ability of being adapted by large consortiums. Furthermore, the uptake of emerging technologies will facilitate rapid progress. If these challenges can be met, there is a real possibility of using circulating miRNAs as effective tools for the clinical management of human diseases.

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Samantha Khoury is a postdoctoral scientist in the Tran Lab. Her PhD (awarded 2018) was focused on discovering novel small RNA biomarkers for the rapid and early diagnosis of head and neck cancers. Her work has already identified a suite of diagnostic serum miRNAs, which are currently being tested in patients with oral cancer.

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Nham Tran is a group leader at the University of Technology Sydney, School of Biomedical Engineering. His lab, which was established in 2013, focuses on miRNAs' and other ncRNAs' potential as biomarkers for cancer diagnosis and prognosis. During his postdoctoral training, Tran published the first study to characterize genome-wide expression of mature miRNAs in different subtypes of head and neck cancer.

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