

miRNAs Expression Profiling, An Exploratory Method for Revealing First-Hand Biomarkers to Predict Disease Progression

Editorial

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Molecular techniques has proven to be a powerful tool to identify reliable predictors of treatment response or disease progression detection and early prediction. Different body cell possess various host expression profiles, and has its own specific indicators to identify different diseases/infection class. Analyzing microRNAs (miRNAs) as a promising host gene plays a critical roles in host interactions with various invaders, including their pathogenesis and host resistance through regulation of post-transcriptional translation or gene expression of related miRNAs, thus acting as a potential biomarkers of infectious diseases. Circulating miRNAs have great potential to facilitate the diagnosis of virus infection, although the discrepancy between the expression levels of intracellular and extracellular miRNAs, that has been observed in certain situations [1]. Circulating miRNAs were found to be extremely stable and protected from RNase mediated degradation within the body fluids, therefore have emerged as candidate biomarkers for several illnesses [2].

In 1993, miRNAs were discovered in the course of an experiment in the nematode; *Caenorhabditis elegans* (*C. elegans*) [3]. Until now, human miRNA family has expanded near to 2000 mature miRNAs (miRBase v21.0; <http://www.mirbase.org>) with approximately 60% of human mRNA could be the targets of miRNA [4]. miRNAs constitutes about 18-22 nucleotides long noncoding RNAs, and playing a vital role in the regulation of gene expression. The production of miRNAs requires some processing phases. Firstly, primary miRNAs (pri-miRNAs) are cleaved by aribo nuclease, called Drosha to produce a precursor miRNAs (pre-miRNAs) which eventually, cleaved by the another ribo nuclease, termed Dicer to produce mature, single stranded miRNAs [5, 6]. At this stages, mature miRNA associate with RNA induced silencing complex (RISC) with Argonaute/EIF2C (AGO) proteins which recognizes their respective target mRNA. miRNA identify their target mRNA through specific base-pairing interactions between the 5' end (termed as "seed" region) of miRNA and a sites within the coding and untranslated regions (UTRs) especially 3' UTR of mRNAs leading to mRNA destabilization. Thus, miR-

NAs inhibits the target gene expression either by mRNA degradation or translational repression [7, 8]. A distinctive nature of miRNAs regulation is that, each miRNA regulates hundreds of different mRNAs, whereas, a single mRNAs are targeted by multiple miRNAs, that are the focus of interest on regulatory networks that determine the cell fate decisions [9].

miRNAs involvement in cancer was first described in 2002 for its regulation in leukemia [10]. While the predictive value might get different among individual miRNAs and expression profiles involvement of multiple miRNAs were expected to play a useful role in tumor classification, diagnosis, and prognosis [11]. It is also thought to be a promising candidate for the next generation-diagnostic biomarkers because of the strong correlation between miRNAs expression patterns and disease status [12, 13]. The level of circulating miRNA is quite stable in healthy people, but down-regulated under certain situations, like physiological changes, inflammation, and cell death [14]. Moreover, profiling of circulating miRNAs may also be utilized for an extended study any association with the mechanism of pathogenesis, tissue damage or cell to cell communications [15]. In addition, circulating miRNAs resist harsh conditions, including RNase digestion, freeze-thawing, boiling, and extreme pH conditions, allowing them to be a promising markers for the non-invasive detection of various diseases [16]. miRNAs stability in body fluids make it a potential marker for sensitive detection by quantitative PCR and non-invasiveness in obtaining samples of body fluids [17].

To serve as a reliable biomarker, standardized methods for serum miRNAs determinations often required. Many methods exist for isolating RNA from serum but accurate measurement of serum miRNAs is challenging due to the low quantity, short length, and high sequence variability [18]. The starting quality of RNA is critical for accurate miRNAs quantification and these circulating miRNAs can be identified in both serum and plasma [19]. Occasionally, a large volume of initial plasma/serum were taken to get the preferable RNA yield for quality down-stream molecular

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analysis [13]. For an effective miRNAs host gene profiling, a good quality control approach with experienced expertise thoughts are required for a successful technical application analysis. Using anti-coagulants during blood collection, EDTA is preferable over citrate and heparin. As both citrate and heparin have an inhibitory effects on real-time PCR as well as next-generation sequencing (NGS) [20]. Archived plasma samples are often discouraged for biomarker identification and significant variation of miRNAs expression in plasma may be contributed by blood-cell specific and platelet specific miRNAs from hemolysis [21].

To get a good miRNAs yield and purity, several extraction process involving phenol-chloroform, guanidinium thiocyanate, ethanol or Silica-based miRNA recovery are employed. But the choice remains to the specific methodology for miRNA extraction from plasma and body fluids depends mainly on initial sample input, techniques of analysis or target spectrums of miRNA species in relation to diseases [22]. For selecting an ultimate analyzing tools, NGS, microarray, and real-time PCR are the methods used to measure miRNAs abundance in expression profiling studies. Though, NGS is not yet practical for serum miRNAs profiling studies due to the requirement of significantly larger RNA input [14] rather, it required by miRNA microarrays is comparable to real-time PCR [23]. However, microarrays offer less sensitivity and require significantly more struggle for data processing when compared with real-time PCR platforms. Thus, real-time PCR is the most commonly used approach in high-throughput expression profiling and quantification [24]. Until today, NGS are the most promising technology for miRNAs profiling and several advantages have been already elaborated elsewhere [21]. Specifically, NGS is extremely sensitive method which also allow a relative quantification and it is capable of differentiating isomiRs from miRNAs [25]. But, the most profound importance of NGS approach to detect and verify novel miRNAs and gene to be get accepted including strong description of its size and structural identity [26]. Though, a very high cost and limited support of computational infrastructure and bioinformatics often discourages its utilizations [22].

miRNAs analysis requires additional quality control and normalization steps that remain unstandardized [27]. Relative quantification is the most common strategy to assess RNA expression differences between two samples. An endogenous control is routinely included with sample input volume for expression normalization [13, 28]. However, the applicability of an endogenous miRNA control may be influenced by disease or treatment creating a biased calculation [29, 30]. Therefore, an extraction control, synthetic *C. elegans* miRNA spike-ins have been used in serum biomarker normalization with much confidence [13].

There are many gaps found on miRNAs findings interpretations, including regulation of miRNA production, specific targets, and mechanisms of active secretion [31]. As for example, circulating liver-specific miRNA-122 was found to be up-regulated on liver injury [28, 32, 33]; whereas, significantly down-regulated during late-stage hepatic cirrhosis [34] and hepatocellular carcinoma [35] due to intracellular down-regulation of miRNA production. Therefore, levels of certain circulating miRNAs may be significantly up-regulated during acute phases, or down-regulated thereafter. Another problem, which might provide an additional difficulty is that most published researchers have designed their experiments to compare a disease group with a healthy group,

rather than including an alternative disease groups. A biomarker may strongly discriminate between a disease group and a healthy group but may also prove to pay an impact with another disease [36]. Therefore, inclusion of an alternate group during the study might solve the issues. Another challenge to the use of miRNAs as markers is that their mRNA targets are not easily identified by computational methods [37] and levels of miRNAs in whole blood are much higher than plasma or serum miRNAs levels [38]. Hence, levels of specific miRNAs in different body fluids were not readily found comparable [39].

Regarding a technical challenge, there is no standardized method for profiling miRNAs found in many published articles. Importantly, sample type, processing, extraction methods etc. vary among reports, and information on the starting fluid volume and particularly the efficiency of extraction were often missing. Investigators may make this information available for easy standardizations and perhaps compare profiles across various patient cohorts [22].

Finally, this editorial tried to describe the potent role of miRNAs as a pertinent host gene that might provide earlier warning signs in various disease prediction, resulting in more effective treatment and improve survival. Thus, multi-center studies with cross-validation are required to generate miRNAs as novel biomarkers as a new diagnostics of diseases.

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