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The Importance of Circulating miRNAs and Its Limitation on the Clinic

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ABSTRACT

MicroRNAs (miRNAs) are endogenous small non-coding RNAs that play key roles in gene expression regulation. Recently it was shown that despite the intracellular function, miRNAs are normally released outside the cells and get into circulation. Circulating miRNAs (cmiRNAs) were described in all body fluids (serum, plasma, saliva, urine, and others). Variations on cmiRNAs levels have been associated with several conditions, such as heart disease, cancer, pregnancy, liver failures and even weight variation. CmiRNAs have been point out as promising biomarkers for early diagnosis, prognosis and therapeutic response predictors for several diseases. Here we describe the importance of cmiRNAs profile variation and the importance of the processing and methodologies to cmiRNA detection, standardization and results from comparison.

INTRODUCTION

MicroRNAs in humans

MicroRNAs (miRNAs) are endogenous small non-coding RNAs that regulate gene expression by mRNA degradation or translational repression [1]. As post-transcriptional gene regulator, miRNAs are important in all cellular process, and variation on their level can have big impact on cells. Moreover, alterations in cells can also cause miRNAs expression variations, affecting cells gene expression. This characteristic made them good biomarkers for specific pathological and biological human conditions. Indeed, miRNAs alterations are common in many human conditions such as inflammation and stroke [2], metabolism [3], immune dysregulation [4], diabetes [5], cancer [6], and others. miRNAs have been explored not only as the biomarker for diagnosis but also for prognosis prediction [7, 8] and even as pharmacological therapeutic targets [9-11]. Furthermore, miRNAs signatures have been reported to be useful for disease classification, such as in cancer [12].

Despite the intracellular function, miRNAs seem to have a role in intercellular communication. In the beginning of the 21st century, it was shown that miRNAs are released outside the cells, from where they can enter into the circulation of human biofluid. Those miRNAs in the circulation are called circulating miRNAs (cmiRNAs). CmiRNAs are present and can be detected in every body fluid, including blood, saliva, tears, urine, among other [13, 14]. Specific cmiRNAs have been shown in many situations, including pregnancy, overweight, health disease such as cancer and heart diseases [14,15].

The cmiRNA function is not completely understood yet, apparently, they have an intercellular communication role, regulating gene expression in distance cells [16], behaving as hormone [17]. CmiRNA may be important to signaling other cells and, in cancer, they can be playing a role in preparing the niche of metastasis [18]. Despite this interesting and promising role, the majority of the studies are focused on cmiRNA as non-invasive biomarkers for human diseases; this must be explained by the facility of sampling them and it can also be a consequence of the high stability of miRNA on fluids.

CmiRNAs expression can be changed by the different situation, including aging [19], exercise [20] and even dietary [21]. Moreover, cmiRNAs have been point out as biomarkers for heart disease [22], aortic stenosis [23], cancer [15], diabetic cardiomyopathy [24], epilepsy [25,26], lupus [27], liver disease [28], among others human disease.

Although cmiRNAs are useful to diagnosis, to monitors disease and therapy response, there are not yet used in clinics. Moreover, there is no universal list of cmiRNA variations for any condition, pointing to the implication that technology and processing.

Methodology implication on circulating miRNAs analysis

The methodology has shown to be very important for cmiRNAs analysis, and it may impact significantly on results, being potentially the main cause for differences on published results of cmiRNAs alterations for pathological and biological conditions [29]. Indeed, there have been some studies showing that not only the origin of the sample, such as saliva, tears, or blood, but also the type of sample pre-processing have big impact on results [30].

Sample origin must be considered when comparing cmiRNAs levels. All cells of our body seem to release miRNAs to the extracellular environments and cmiRNAs can potentially be found in any biofluids. However, a not completely elucidate but a specific mechanism regulates cmiRNAs releases. Indeed, when comparing cells' miRNAs expression to cmiRNAs expression there is not always a complete match, pointing to a specific regulation mechanism for the release of miRNAs to the extracellular environmental [31]. Different pattern of cmiRNAs has been shown to exist between diverse samples types. In this context, special attention needs to be paid when comparing cmiRNAs results for a specific condition once that other cellular processes may be influencing cmiRNAs levels.

Furthermore, sample processing can also influence cmiRNAs patterns due to the release of intracellular miRNAs consequences of others cellular mechanism [30]. For instance, serum and plasma can present different cmiRNAs profiles mainly due to platelets and coagulation factor, Indeed, there is a complex process with the release of differences molecules during those events [32]. Platelet activation can also lead to a variation in cmiRNAs levels [33]. Furthermore, hemolysis and cell blood contamination are also important [34], cell lysis may cause a variation on cmiRNAs by the release of the intracellular miRNAs of the lysates cells.

RNA extraction protocols and storage can influence on RNA quality. The quality of RNA is also fundamental to guarantee good results, and are necessary to consider when comparing data. Indeed, several protocols have been proposed for studying cmiRNA in human disease, however, even among them there is variation, including centrifugation and RNA extraction protocols, that can impact on cmiRNA levels analysis [35], [36].

Lastly, data analysis is also important, can also influence results, and can compromise data comparison. Normalization is one of most controversial aspect of miRNAs studies, and several miRNAs have been proposed to use as endogenous control, however, there is no consensus yet, for example in urinary samples it has been proposed to used miR-16 [37] while miR-6090 and miR-4516 are suggested as endogenous controls on coronary artery disease [38]. This is one of major problem when analysis cmiRNA expression. There is no universal endogenous control and this can implicate in different results when comparing. Other methods that include incorporation of exogenous miRNAs such as spike and normalization by samples volume were also analyzed, and are in most cases not recommended [35].

SUMMARY

Despite the potential usability of cmiRNAs as biomarker for detection, identification, monitoring disease progression, and evaluation of therapy response for many pathological human conditions there is still a lack of patronization of the methodology used for cmiRNAs to be able to compare different results. Indeed, there is a huge number of factor that may alter the cmiRNAs level between cohorts and studies. Special attention must be paid to methodology when comparing result from different groups. The methodology differences affects tremendously results, and can limits the use of cmiRNAs, delaying health and technological improvements.

Conflict of interest

There is no conflict of interest.

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