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Challenges in the Analysis of Micro RNAs from Liquid Biopsy as Epigenetic Biomarkers in Clinical Samples

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Introduction

Advances in precision medicine, with better diagnostic and treatment strategies, are the hope to improve the management of diseases, comorbidities and mortality. For this purpose, biomarker research, especially based on liquid biopsies, has increased in recent years.

We recently defined an epigenetic biomarker as "any epigenetic mark or altered epigenetic mechanism" that can: (1) be measured in the body fluids or tissues; (2) detect a disease; (3) predict the outcome of a disease and future comorbidities (prognostic); (4) respond to therapy (predictive); (5) monitor responses to therapy or medication (therapy monitoring); and (6) predict risk of future disease development (risk). Therefore, miRNAs are potential biomarkers that fulfill most of these requirements.

In this context, epigenetic dysregulation and in particular microRNAs (miRNAs) play a central role in the initiation, progression and treatment response of human diseases because of their dynamic nature.

Advantages of miRNA Analysis in Liquid Biopsy

There are plenty of advantages of epigenetic biomarkers compared to genetic biomarkers, one of them being that the former provide vital information about gene function in individual cell types, and specific phenotypes [1,2]. For example, altered transcriptional profiles of miRNAs are a hallmark of several human diseases [3]. Such differential miRNA alterations have been taken advantage of to provide new biomarkers to improve clinical decisions in medicine.

It is worth mentioning that miRNAs are responsible for the fine-tuning of fundamental cellular activities and human disease development [4]. For this reason, miRNAs can fill clinical research gaps by incorporating new, relevant information regarding epigenetic control on specific genetic programs and, in turn, on the molecular pathways underlying pathological processes.

A relevant feature of miRNAs is their high stability in samples of compromised quality (e.g. thawed and FFPE tissues) [5], as well as the fact that they can be detected efficiently in a wide array of biospecimens (e.g. cells, fresh and frozen tissues, FFPE, etc.) [6]. They can also be detected in body fluids such as saliva, blood (plasma and serum), sputum, tears, breast milk, and cerebrospinal fluids [7,8].

Additionally, it has been demonstrated that miRNAs are stable in archived serum samples frozen at -25°C for at least 40 years [9] and at -20°C for at least 2-4 years [10]. Because of these properties, miRNAs hold great promise as minimally invasive diagnostic and prognostic biomarkers.

Challenges in the Analysis of miRNAs on Liquid Biopsy

Due to the intense research activity in this field, an increased number of miRNAs are being proposed as biomarkers but there is still a long way ahead to convert them into reliable tools for diagnosis and prognosis.

The detection of miRNA profiles in several biofluid samples poses a challenge in many ways. For example, biofluids contain very low amounts of RNA, and miRNAs are a small fraction of the total RNA. In addition, biofluids and the collector tubes used for sampling can contain inhibitors of enzymatic reactions (e.g. heparin) used in some detection methods, which could mask the subsequent detection of subtle changes in the miRNA expression profile.

Four critical factors can alter the interpretation of the results obtained after analyzing miRNAs: 1) the source of miRNA (cellular or extracellular origin); 2) the isolation/purification method; 3) the detection method and 4) the reference or normalization method used to interpret the results.

On the one hand, blood is one of the most common samples used in clinical research and diagnostics, possibly due to the fact that it is a readily available tissue and there are a lot of blood derivative samples stored in repositories or biobanks, making it easy to access. Furthermore, isolating miRNA from serum or plasma is relatively straightforward. The miRNAs detected in biological samples may be of cellular or extracellular origin [11], and both may be relevant in terms of biomarker discovery. On the other hand, sometimes the regulatory genetic mechanisms in which these miRNAs are involved have not been fully clarified.

Although miRNAs are highly expressed in blood cells, the level of plasmatic miRNA can be altered significantly by the various extents of hemolysis [12]. For this reason, hemolysis is one of the obstacles to overcome if the goal is to analyze the extracellular miRNA profile in biofluids, which are also susceptible to many pre-analytical variables. The clinical samples derived from pathological body fluids may contain not only sick cells but also large proportions of normal cells that can also release altered levels of the biomarker of interest, therefore complicating the interpretation of the results. Thus, when monitoring the levels of miRNAs in consecutive samples, e.g. during treatment monitoring, changes in miRNA signatures or levels could reflect a mere variation in the composition of the broken cell types within the samples instead of changes in the miRNA profile of the said biomarker. In addition, miRNAs can be released to the blood via a) exosomes, b) microvesicles, c) apoptotic bodies, d) high density lipoprotein (HDL) and e) AGO protein complex [13]. Therefore, when analyzing miRNAs, one must take into account which miRNA is to be isolated and in function of that, choose the appropriate method or kit for miRNA purification.

Nonetheless, there are many recommendations for overcoming these handicaps. For instance, it is usually advisable to use plasma samples rather than total blood samples and it is also better to choose a group of miRNA comprising an miRNA-signature, rather than only one miRNA. Preventing lysis of cells during all steps related to sample preparation is also recommended, preferably using EDTA and citrate as anticoagulants, since heparin can inhibit downstream enzymatic steps such as cDNA synthesis and PCR.

The miRNA isolation and purification steps are critical processes, and many aspects must be considered in order to make it feasible to use an miRNA as a biomarker. There are several protocols and commercial kits for miRNA purification that isolate free circulating miRNAs, protein-bound miRNAs, microvesicle-associated miRNAs or total miRNA from several biofluids such as serum/plasma, urine and others [14], but they still have certain limitations to overcome in order to successfully perform biomarker measurement.

Likewise, there are several platforms used for profiling miRNAs in biofluids based on NGS, arrays and RT-qPCR. NGS is the method of choice if the main goal is to discover novel miRNA sequences, or study isomiRNAs or miRNA editing. In turn, arrays offers the flexibility to perform miRNome analysis, while qPCR enables miRNA profiling and validation of a subset of miRNAs, and is well suited for accurate differential expression analysis of a defined set of miRNAs [15]. Besides those current technologies for miRNA analysis, various nanotechnology-based approaches are appearing, such as miRNA biosensors, which in the near future will revolutionize miRNA detection and quantitation [16].

Although miRNA detection methods are widely studied, data normalization is yet unresolved, and choosing the appropriate miRNA for data normalization of differentially expressed circulating miRNAs is of special relevance to accomplishing better results [17,18]. At the present time, the identification of universal miRNA normalizers for a wide array of human pathologies seems unlikely, although using algorithms for data normalization (e.g. NormFinder, GeNorm, CV score and DataAssist) may help with this issue [18]. Therefore, the strategy should be based on the adoption of standardized methods to select disease-specific miRNA normalizers in plasma samples. Fortunately, the development of new techniques that allow absolute quantitation, such as droplet digital PCR (ddPCR), will help. In fact, the adaptation of new technologies and methods to a clinical laboratory environment may ensure the adoption of epigenetic biomarkers into the diagnostic process.

Conclusions

Although great effort is being made to implement protocols for effective sampling of biofluids and to develop new techniques to ascertain miRNA profiles related to pathological conditions in such biofluids, only a low number of reliable epigenetic biomarkers are currently being considered by clinicians. However, with technological advances, panels of miRNAs could be robustly, routinely, accurately, and inexpensively profiled in near future, to provide detailed information for patient diagnosis, prognosis, stratification and treatment decision and monitoring. miRNA

have emerged as diagnostic targets in precision medicine, so we consider that US Food and Drug Administration (FDA) will approve new tests, methods and procedures for miRNA-based diagnostic in the near future.

Reference

1 Lee J, Choi KJ, Moon SU, Kim S (2016) Theragnosis-based combined cancer therapy using doxorubicin-conjugated microRNA-221 molecular beacon. Biomaterials 74: 109-118.

2 García-Giménez JL, Seco-Cervera M, Tollefsbol TO (2017) Epigenetic biomarkers: Current strategies and future challenges for their use in the clinical laboratory. Crit Rev Clin Lab Sci 2017: 1-22.

3 Garcia-Gimenez JL (2015) Epigenetic Biomarkers and Diagnostics.

4 Shu J, Silva BVRE, Gao T, Xu Z, Cui J (2017) Dynamic and Modularized MicroRNA Regulation and Its Implication in Human Cancers. Sci Rep 7: 13356.

5 Peiro-Chova L, Pena-Chilet M, Lopez-Guerrero JA (2013) High stability of microRNAs in tissue samples of compromised quality. Virchows Arch 463: 765-774.

6 García-Giménez JL, Ushijima T, Tollefsbol T (2015) Epigenetics Biomarkers and Diagnostics. In: García-Giménez J, ed. Epigenetic Biomarkers and Diagnostics. London: Academic Press; 2015: 680.

7 Dumache R, Ciocan V, Muresan C, Rogobete AF, Enache A (2015) Circulating MicroRNAs as Promising Biomarkers in Forensic Body Fluids Identification. Clin Lab 61: 1129-1135.

8 Weber JA, Baxter DH, Zhang S (2010) The microRNA spectrum in 12 body fluids. Clin Chem 56:1733-1741

9 Rounge TB, Lauritzen M, Langseth H, Enerly E, Lyle R, et al. (2015) microRNA Biomarker Discovery and High-Throughput DNA Sequencing Are Possible Using Long-term Archived Serum Samples. Cancer Epidemiol Biomarkers Prev 24: 1381-1387.

10 Grasedieck S, Schöler N, Bommer M (2012)Impact of serum storage conditions on microRNA stability. Leukemia 26: 2414-2416.

11 Etheridge A, Lee I, Hood L, Galas D, Wang K (2011) Extracellular microRNA: a new source of biomarkers. Mutat Res 717: 85-90.

12 Kirschner MB, Edelman JJB, Kao SC-H, Vallely MP, van Zandwijk N, et al. (2013) The Impact of Hemolysis on Cell-Free microRNA Biomarkers. Front Genet 4: 94.

13 Sohel MH (2016) Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and Challenges. Achiev Life Sci 10: 175-186.

14 Moret I, Sánchez-Izquierdo D, Iborra M (2013) Assessing an improved protocol for plasma microRNA extraction. Hawkins SM, edt. PLoS One 8: e82753.

15 Git A, Dvinge H, Salmon-Divon M (2010) Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. RNA 16: 991-1006.

16 Kilic T, Erdem A, Ozsoz M, Carrara S (2018) microRNA biosensors: Opportunities and challenges among conventional and commercially available techniques. Biosens Bioelectron 99: 525-546.

17 Tiberio P, Callari M, Angeloni V, Daidone MG, Appierto V (2015) Challenges in Using Circulating miRNAs as Cancer Biomarkers. Biomed Res Int 2015: 1-10.

18 Marabita F, de Candia P, Torri A, Tegnér J, Abrignani S (2016) Normalization of circulating microRNA expression data obtained by quantitative real-time RT-PCR. Brief Bioinform. 2016;17(2):204-212.