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Review article

microRNAs for peripheral blood fraction identification: Origin, pathways and forensic relevance

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ABSTRACT

microRNAs (miRNAs) are small non-coding RNAs, with a length of 18 to 24 nucleotides that play a regulatory role in several cellular processes. Since their discovery, they have been identified in cells, tissues, organs, and body fluids and their potential as molecular biomarkers for the diagnosis of various pathologic conditions has been explored. However, little is known about the origin of the extracellular miRNAs and what factors influence the levels of circulating miRNAs. This information could help the refinement of miRNAs as more effective biomarkers. Additionally, the identification of the origin of miRNAs may prove to be very useful in the association of particular miRNAs with specific pathologies.

This review aims to gather information concerning the origin of miRNAs in plasma and serum, as well as to assess their potential to be use as biomarkers for these peripheral blood fractions.

Contents

1. Introduction

The different fractions of peripheral blood, which include plasma, serum and blood cells — leucocytes or white blood cells (WBCs), erythrocytes or red blood cells (RBCs) and platelets — differ from each other based on several factors [1]. Both serum and plasma contain hormones, glucose, electrolytes, antibodies, antigens and other particles. Plasma corresponds to the cell-free supernatant resulting from centrifuged

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blood collected in the presence of an anticoagulant [2]. On the other hand, serum is the cell-free supernatant resulting from centrifuged blood collected in the absence of an anticoagulant, and therefore, does not contain most of the clotting factors, such as the fibrinogen, and being unable to clot [2].

Recently, it has been accepted that the fractions of peripheral blood also differ in their microRNA (miRNA) content. miRNAs are short non-coding RNA, with 18 to 24 nucleotides in length that play a regulatory role in several cellular processes [3]. miRNAs are transcribed from the genomes of nucleated cells by RNA polymerase II or III and these primary miRNA transcripts (pri-miRNAs) are then modified by the addition of a 5′ cap and a 3′ poly-A tail [4]. Subsequently, the pri-miRNAs are processed first in the nucleus by the RNase III enzyme Drosha and later in the cytosol by the RNase III enzyme Dicer [4]. This processing of pri-miRNAs originates initially a miRNA precursor (pre-miRNA) with approximately 70 nucleotides in length, and finally a smaller and mature double-stranded miRNA of 18–24 nucleotides [4]. These molecules can be found not only on the intracellular compartments, but also in the extracellular microenvironment, namely in serum, plasma and urine [5]. However, there is little information concerning the origin of circulating miRNAs (both in healthy and diseased individuals) and what factors may influence their levels [6].

2. Fractions of peripheral blood and circulating miRNA origin

The extracellular miRNA group is diverse, considering that some miRNAs can be packaged into apoptotic bodies, microvesicles (MVs), or high density lipoprotein (HDL) particles, while others are exclusively complexed with argonaute (AGO) proteins [7]. The vesicle group comprising both shedding vesicles and exosomes, is frequently designated as MVs [8]. Apoptotic bodies are particles with a size that can range between 1 to 4 μm and that remain after programmed cell death initiation [7]. Shedding vesicles are heterogeneous vesicles (100–1000 nm) that are released by outward budding and fission of the plasma membrane, whereas exosomes are smaller vesicles (30–100 nm) that are released following the fusion of endosomal-derived multivesicular bodies with the plasma membrane [4,8]. miRNAs have been identified in exosomes and microvesicles recovered from different body fluids, such as plasma, serum, saliva and urine [4,9].

According to Arroyo et al., the majority of circulating miRNAs are protein bound, rather than associated with vesicles [10]. Turchinovich et al. corroborate this information, showing that extracellular miRNAs are mainly exosome/microvesicle free and are associated with AGO proteins [11]. When blood plasma is analyzed the situation is similar, since 90–95% of the totality of miRNAs in cell-debris-free blood plasma is circulating in an AGO-protein-bound form, while the microvesicle-associated miRNAs are a minority [7,10,11]. On the other hand, Gallo and co-workers found that the majority of miRNAs detectable in the serum of healthy individuals and systemic lupus erythematosus patients are primarily in exosomes [9]. This divergence in results supports the need to elaborate further studies. Moreover, although several miRNAs were detected in purified fractions of HDL from human plasma, their proportion in blood plasma is still unknown [7,12]. Regarding the miRNAs packaged into apoptotic bodies, there is a lack of information concerning their contribution to the circulating miRNA population. Furthermore, the fact that the size of apoptotic bodies is similar to that of cell debris and blood platelets and that the protocols frequently used in extracellular miRNA investigation involve the elimination of cell debris and apoptotic bodies, this may cause an overlook of the cell-free miRNA in these fractions [7].

In the peripheral blood, since blood cells have a vast contact with plasma and serum, they may contribute in an extensive way to the extracellular miRNA content in these fluids [7]. In fact, in a study where miRNAs from serum and blood cells of healthy subjects were sequenced, the majority of miRNAs (91 in 101 miRNAs tested) were detected in both serum and blood cells, while only six miRNAs were exclusively detected in serum (miR-100, let-7c, miR-184, miR-23a, miR-339-5p and miR-923) and four in blood cells (miR-221, miR-301a, miR-335 and miR-576-3p), suggesting that under normal conditions most serum miRNAs are derived from circulating blood cells [13]. This study also concluded that the expression levels of serum miRNAs among healthy individuals were consistent [13]. However, it would have been also interesting to examine and compare the expression levels of serum circulating miRNAs and blood cells miRNAs, in order to access potential alterations between the miRNA expression patterns in these fractions of peripheral blood.

Likewise, through a comparative analysis of the miRNA pattern in healthy individuals and in patients with various diseases that exhibit alterations in blood cell counts (such as squamous cell carcinoma of the tongue, acute myeloid leukemia, metastatic esophageal cancer, chronic myelogenous leukemia, autoimmune hemolytic anemia, multiple myeloma, metastatic renal cell carcinoma and glioblastoma, among others), Pritchard et al. observed that blood cells are a major contributor to circulating miRNA [6]. Moreover, Wang et al. reported that the difference between serum and plasma miRNA expression levels exhibited some associations with platelets-derived miRNAs, therefore indicating that platelets may release miRNAs to serum and also that the coagulation process may affect the spectrum of extracellular miRNA in blood [14]. However, Turchinovich and Burwinkel did a comparative analysis of AGO1- and AGO2-associated miRNAs in blood plasma and the whole blood pellet, verifying that the AGO-specific miRNA profiles in blood cells diverged significantly from miRNA profiles in plasma, thus indicating that most circulating miRNAs are likely to derive from non-blood cells under normal conditions [15]. Since these studies came to different conclusions, it is imperative to replicate these studies, in order to better understand the miRNA origin in plasma and serum.

Other factors may contribute to miRNA expression in plasma, for example, Chen et al. validated the possibility to analyze the miRNA expression using serum and plasma without any RNA extraction or serum filtration method, what supports the hypothesis that lysed cells might also be a contributor to the plasma miRNA composition [13,16].

Additional evidences, like the endothelial origin of miRNAs in plasma and serum, cannot be dismissed, bearing in mind that the endothelium tissue establishes a close contact with these fluids [17]. In fact, Williams et al. profiled the expression levels of circulating miRNA from human blood samples and compared with that of the endothelial tissue. Their results demonstrated that the majority of circulating miRNA molecules originate not only from blood components, but also from endothelial cells [18]. Furthermore, this study also detected tissue specific miRNAs in blood samples, namely, from liver and gut. Tissue-specific miRNAs, like miR-122 (liver), miR-133a (muscle), miR-208a (heart), and miR-124 (brain), have also been identified in blood plasma, indicating that other organs might also contribute to the circulating miRNA population [19–22].

3. Circulating plasma and serum miRNAs

For most miRNAs present in plasma and serum their specific cellular origin is unknown, however the pattern expression of a vast number of miRNAs is established, making it possible to determine its probable source (Fig. 1).

Here, we further explore the possibility of an endothelial, blood cell and organ origin of some miRNAs present in plasma and/or serum and suggest the hypothesis that some miRNAs could originate from both endothelial and blood cells (Table 1). Once the miRNAs released from blood cells into plasma and serum are determined it would be interesting to study the alterations in the miRNA expression pattern among individuals with blood-related diseases in order to assess possible associations. We may consider that this can also have forensic implications, since it can be useful in the identification of the donor of a body fluid. For example, in a forensic investigation where there is only access to the suspects clinical files, and is not possible to obtain a blood sample