Mini-Review

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Peripheral blood microRNAs: A novel tool for diagnosing disease?

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Summary **Peripheral blood microRNAs (miRNAs) are endogenous, noncoding small RNAs present in blood. Because of their size, abundance, tissue specificity, and relative stability in peripheral circulation, they offer great promise of becoming a novel noninvasive biomarker. However, the mechanism by which they are secreted, their biological function, and the reason for the existence of extracellular miRNAs are largely unclear. This article describes advances in the study of the mechanism of origin and biological function of extracellular miRNAs along with approaches adopted by research and questions that remain. This work also discusses the potential for peripheral blood miRNAs to serve as a diagnostic tool.**

Keywords: Peripheral blood miRNAs, diagnosis, biomarker, biological function

1. Introduction

The traditional method for diagnosing disease was to find pathological tissue. This method was highly specific but lacked sensitivity and involved greater harm to the patient. Therefore, the pressing need was to find a type of noninvasive and highly accurate method of diagnosing disease. MicroRNAs (miRNAs) are endogenous noncoding RNA molecules of 21-23 nucleotides that negatively regulate gene expression by binding to sites in the 3' untranslated regions of target mRNAs, causing a degradation or blockade of the translation. Since their discovery in the early 1990s, miRNAs have been found to play important regulatory roles in a wide range of biological and pathological processes. In recent years, rapid advances in sequencing techniques have greatly improved the sensitivity of their detection, and many miRNAs have been noted in serum and plasma. Studies have found that miRNAs are highly stable in peripheral blood containing ribozymes and some miRNAs, and their levels differ significantly in patients with different diseases (*1,2*). Furthermore, levels of expression of specific peripheral blood miRNAs are correlated with certain clinicopathological variables and could serve as a novel diagnostic biomarker for detection of disease and could be used clinically to monitor disease progression (*3-8*). However, some researchers began to question their diagnostic value given that peripheral blood miRNAs have not been found to play a functional role in the etiology or progression of disease (*9*). There are doubts about whether levels of expression of peripheral blood miRNAs can be regarded as diagnostic targets and the diagnostic value they may have.

2. miRNAs in peripheral blood

As an existing form of miRNAs, peripheral blood miRNAs come directly from exosomes, which are vesicles 30-100 nm in diameter. Since Johnstone *et al*. discovered that exosomes perform a number of activities (*10*), as exemplified by the reticulocyte plasma membrane in sheep reticulocytes cultured *in vitro*, exosomes have been gradually emerged from the shadows. Researchers found that purified exosomes contain functional miRNAs and originate from endocytic compartments that are released by many cell types (*11,12*). A study concerning the mechanism of exosome secretion found that miRNAs increased through overexpression of neutral sphingomyelinase 2 (nSMase2) (*13*). Decreasing the activity of nSMase2 with a chemical inhibitor, GW4869,

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and specific small interfering RNA resulted in the reduced secretion of miRNAs, so exosomes are released *via* ceramide-dependent secretion. Moreover, a study examining mammary epithelial cells releasing miRNAs revealed that mammary epithelial cells typically release similar exosomes (*14*). That study measured the level of expression of CD63 and CD81, endosomal marker proteins, and the authors considered extracellular miR-16 to be a surrogate marker for the abundance of endosomal miRNAs.

In addition, levels of miRNAs and exosomes in peripheral circulation are correlated with breast cancer (*5*), osteoarthritis (*15*), lung cancer (*16,17*), and ovarian cancer (*18*). The relative expression of some miRNAs is closely associated with clinicopathological features of cancer, such as their histologic grade and pathology. In short, these studies have highlighted the potential for use of exosomal miRNAs profiles as diagnostic biomarkers of disease through noninvasive testing. Nonetheless, cells selectively release miRNAs. Pigati *et al*. found that the bulk of miR-451 and miR-1246 produced by malignant mammary epithelial cells was released but that most of the miRNAs produced by non-malignant mammary epithelial cells were retained (*14*). In addition, Tanaka *et al*. found a high level of miR-92a expression in tissue samples from patients with acute leukemia but a reduced level of miR-92a in plasma (*19*). Lodes *et al*. also found that the expressional profiles of serum miRNAs did not directly correspond to tissue profiles (*20*). Therefore, some types of cells selectively release miRNAs, and other pathways of miRNAs secretion must exist.

A study of the secretary pathway of let-7, a tumor-suppressive miRNAs, that targeted oncogenes such as *RAS* and *HMGA2* found that not all types of cells secreted exosomes (*21*). Moreover, another study did not detect exosomal miRNAs in serum from normal controls (*20*). However, a variety of tissue-derived miRNAs exist in the peripheral circulation of healthy people (*22*). So where do these miRNAs come from? Some researchers believe that cell death or cell injury is the mechanism of miRNAs release (*7,23,24*). Alternatively, the release of miRNAs in exosomes is correlated with house-cleaning, whereby cells release damaged components and other cellular components into the environment (*25*). Regardless, none of the hypotheses can sufficiently explain the mechanism of miRNAs release. Therefore, the mechanisms involved in miRNAs release and whether different cells have the same mechanism are questions that still need to be determined.

3. Functions of peripheral blood miRNAs

As an important regulator in the post-transcriptional control of gene expression, miRNAs are involved in major biological processes of cancers, including metastasis, differentiation, apoptosis, and proliferation. In the blood, cellular interactions between erythrocytes, leukocytes, platelets, and endothelial cells are regulated by complex mechanisms that involve multiple molecules. Interestingly, exosome-derived miRNAs are one such molecule. Circulating miRNAs exist in the form of exosomes (*26*), which play an important role in intercellular communication, and mature miRNAs can be transferred between circulating cells through exosomes (*12*).

Many recent studies have noted that miRNAs are important participants in erythropoiesis (*27-30*), lymphopoiesis (*31-33*), the modulation of innate immune response (*34-36*), adaptive immune response (*37-39*), and the differentiation of leukocytes (*40*) and dendritic cells (DCs) (*41,42*). These findings show that exosomal miRNAs are crucial to the functioning of blood cells, and miRNAs could be manufactured into drugs to correct for autoimmune diseases or other diseases related to blood cells.

As miRNAs transporters, exosomes contain inactive miRNAs. Instead of a single messenger, exosomes can deliver multiple miRNAs at one time to neighboring cells and simultaneously suppress related genes (*43*). Thus, exosomes are a potential way to treat complex and uncontrollable diseases. However, the mechanism by which exosomes bind to the cellular surface and exosomal miRNAs enter into cells is not known. Also unknown is whether exosomes selectively bind to neighboring cells. Other pathways through which miRNAs enter cells need to be determined.

4. The potential use of peripheral blood miRNAs as a diagnostic tool

To deduce miRNAs involved in the progression of non-small-cell lung cancer (NSCLC), one study examined exosomes, another examined serum, and the third examined plasma (*16,44,45*). The three studies came up with different results. Other studies found that different techniques lead to different results (*46,47*). This suggests that appropriate specimens and techniques must be used for results to be generalizable and valid.

Whole blood, serum, and plasma can be used to study peripheral blood. Given its relation to erythrocytes, leukocytes, and platelets, whole blood is often chosen when studying immune diseases, inflammatory diseases, or coagulation factor deficiencies, while other studies choose serum or plasma. In actuality, the best specimens are ones that characterize the status of disease; in most diseases, exosomes are the best choice because their origin is known.

The qualitative and quantitative analysis of miRNAs is essential. Microarrays are often used to that end, as are quantitative real-time polymerase chain reaction (qRT-PCR) systems. Real-time PCR using the TaqMan Array Human MicroRNA panel is a novel and practical means of high-throughput investigation of serum RNA samples (*48*). Lodes *et al*. invented a type of microarray platform

that enables the simultaneous analysis of all human microRNAs *via* either fluorescent or electrochemical signals (*20*). This platform could easily be redesigned to include newly identified miRNAs without the need for amplification. Further developments have taken place. For example, Lusi *et al*. manufactured an electrochemical genosensor that is able to directly detect miRNAs without the need for PCR and a labeling reaction; their technique is simple, fast, and ultrasensitive (*49*). Heneghan *et al*. developed a reverse-transcription qRT-PCR assay that can detect circulating miRNAs in serum without RNA isolation (*9*). To improve accuracy, Moltzahn *et al*. invented a multiplex qRT-PCR technique involving purification of multiplex PCR products followed by uniplex analysis on a microfluidic chip to evaluate 384 human miRNAs (*3*).

5. Conclusion

Systems biology is defined as a comprehensive quantitative analysis of the manner in which all of the components of a biology system interact functionally over time (*50*). At the molecular level, the focus of systems biology is to determine the functioning of key molecules in cell signal transduction and gene regulation networks. miRNAs are one class of small, non-coding regulatory molecules, and they play an important role in diverse biological processes such as development, cell proliferation and differentiation, apoptosis, oncogenesis, metabolism, angiogenesis, and inflammation. Therefore, studying the functions of miRNAs is essential to understanding the mechanism of disease and to perceiving the internal workings of biosystems.

As an existing form of miRNAs, peripheral blood miRNAs are encased in exosomes where they are protected from enzymatic degradation. They bind to neighboring cells to regulate the expression of target genes. Most recent studies on peripheral blood miRNAs focused on whether peripheral blood miRNAs can serve as a novel noninvasive biomarker. Surprisingly, almost all found that some (tissue-specific or tissue-nonspecific) circulating miRNAs were correlated with the development and progression of disease. However, the origin and functioning of peripheral blood miRNAs are unclear. miRNAs may exist in another form when they act in peripheral blood.

Peripheral blood miRNAs offer promise in the area of prenatal diagnosis. Evidence has revealed that some placental-specific miRNAs are consistently detected in maternal serum or plasma (*2,51*). Further research demonstrated that chorionic villous trophoblasts continuously released placenta-specific miRNAs into maternal circulation *via* exosomes (*52*). The level of expression of placenta-specific miRNAs in maternal peripheral blood is closely related to pre-eclampsia (PE) (*3*), congenital heart defects (CHD) (*53*), and fetal growth restriction (*54*).

In addition, some specific miRNAs have better sensitivity and specificity when distinguishing healthy specimens from those with disease, such as the most common forms of cancers: breast (*6,7,55*), prostate (*47*), lung (*16,44*), and colorectal cancer (*56,57*). A study of the potential for miRNAs to serve as a biomarker in drug-induced liver injury found that specific miRNAs species exhibited dose- and exposure duration-dependent changes in the plasma that parallel the histopathology of liver degeneration and levels of serum aminotransferase, an earlier biomarker for liver injury, but their changes can be detected significantly earlier (*22*).

In conclusion, recent studies have shown that tissue-specific miRNAs in peripheral blood may be a potential biomarker because they are noninvasive and reproducible and also because they are accurate (sensitive and specific) and predictable. Therefore, peripheral blood miRNAs may offer a better tool for the diagnosis of disease, though some issues remain.

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