Circulating MicroRNAs as Novel Disease Biomarkers: Can They be Applied in Daily Clinical Practice?

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Introduction

Just over a decade ago, microRNAs (miRNAs) were discovered in mammals [1] to constitute a large family of short noncoding RNA sequences, approximately 20-22 nucleotides, synthesized in the cell nucleus, through a complex multi-step biosynthetic process, starting from RNA polymerase II; it is estimated that the human genome contains more than 2500 mature miRNAs [2,3].

These nucleotides modulate gene expression, by binding the 3′-untranslated region of target messenger RNA (mRNA), both degrading mRNA and inhibiting protein translation; miRNAs regulate a wide range of biological processes as cell differentiation, proliferation and development, cell-to-cell communication, cell metabolism and apoptosis [3,4].

miRNAs are contained in tissue cells but they are also detectable in extracellular sites, as plasma and other body fluids in which they are carried within small membrane vesicles (exosomes), in the form of high-density lipoprotein complexes, or complexed to carrier proteins (argonaute-2 proteins); in 2008, miRNAs were also detected in platelets, erythrocytes, and nucleated blood cells [5-7]. Extracellular miRNAs in exosomes may be transferred to other cells, altering gene expression and changing the functional effects of receivers [8-10].

There is evidence that miRNAs may have a role in molecular mechanisms linked to cellular pathways of certain diseases, as viral infections, cancer, diabetes and cardiovascular disease [3,4,11,12].

Literature evidences and Discussion

In 2002, for the first time, the link between miRNAs and cancer was reported in patients with B-cell chronic lymphocytic leukemia; in these patients was found a downregulation of miR-15a and miR-16-1 [13]. Subsequently, a lot of studies have proven that expression of some miRNAs is closely correlated with cancer development and progression; indeed, it has been shown that some miRNAs may function as oncogenes or tumor suppressors [14-16].

Significant correlations were found between a lot of miRNAs and several types of human cancer as colorectal cancer, pancreatic adenocarcinoma, bladder cancer, lung cancer and malignant pleural mesothelioma, urinary and prostate cancer, breast cancer, hematologic malignancies, glioblastoma and others, so that it was suggested that these circulating nucleotides may be used for early diagnosis, staging, follow up, assessment of therapeutic responses and therapy outcomes in cancer patients [17]. Thus, as a result, the study of miRNA has become a rapidly emerging field in oncology and the detection of miRNA expression is a very important first step in miRNA exploration. Currently, conventional cancer biomarkers commonly utilized in clinical practice as, carbohydrate antigens, oncofetal antigens, hormones, enzymes, tissue polypeptide antigen and others, are usually employed in follow up of cancer patients; however, they have low specificity and sensitivity so that monitoring disease is the most common clinical use of these biomarkers [18-21]; thus, miRNA detection could be a new effective tool in the clinical management of cancer. The main miRNAs more related to solid malignancies are: miR-21, miR-29c, miR-92a, miR-125, miR-126, miR-200b-c and others [17].

In the past few years, the potential role of different miRNAs in cardiovascular diseases has been widely recognized. In several studies it has been proved that some heart-specific miRNAs (miR-208, miR-499, miR-1 and miR-133) are consistently increased in plasma of patients with acute myocardial infarction (AMI) within few hours after the onset of infarction [22-24], thus it has been hypothesized that miRNAs might be used to detect and monitor myocardial injury. Wang et al. have shown that miR-208a may have advantages over classic cardiac biomarker troponin I (cTnI) in the early stages of AMI, because this miRNA achieves its peak before cTnI. It was found that miR-208a might be detected in plasma of all patients within 4 hours of the onset of symptoms, whereas cTnI was only detected in 85% of patients at this early stage. In this regard, it has been suggested a faster leakage of miRNAs than cTnI from damaged cardiomyocytes; cTnI is mainly bound to myofibrils whereas miRNAs are probably bound to protein complexes in the cytosol and so the latter allows a faster release from damaged cells [25,26].

Some studies have considered a possible role of circulating miRNAs as biomarkers for atherosclerotic disease; several downregulated and upregulated miRNAs were found in plasma of patients with atherosclerotic disease. For instance, miR-126, miR-92a, miR-145, miR-155 and miR-17, were abundantly expressed in the vessel wall, in endothelial cells, in vascular smooth muscle cells and in inflammatory cells of atherosclerotic disease patients [27]. Other researches have shown that miR-33a,b, miR-92a, miR-126 and others might play a determinant role in several processes involving regulation of lipid biosynthesis, lipoprotein metabolism, immune responses, endothelial cell biology and vascular function [28]. miRNAs have also been linked to various aspects of vascular remodelling and it has been hypothesized a potential role of some miRNAs, as miR-126, miR-155 and microRNA gene clusters 17-92, 23/24/27, 143/145 and 14q32, in regulating multiple vascular remodelling processes, including maladaptive processes of atherosclerosis, vascular restenosis and aneurysm formation [29].

The common circulating biomarkers for cardiovascular disease are specific proteins, such as troponins and natriuretic peptides; however detection of these current biomarkers is usually based on antibodies, which may exhibit cross-reactivity with other proteins. Circulating miRNAs might offer greater advantages in terms of sensitivity and specificity because they are stable, their sequences are evolutionarily

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conserved, their expression is often tissue or pathology specific and because they are detected by highly sensitive and specific assay [12]. Unfortunately, most of the clinical studies on miRNAs were limited by a relatively small number of enrolled patients; therefore, large-scale trials are needed to determine the potential role of circulating miRNAs as biomarkers in diagnosis and prognosis of patients with cardiovascular diseases. The need to undertake further large-scale and long-term clinical studies becomes even stronger when we consider that clinical researches on miRNAs have aroused interest not only in their possible future diagnostic role but also with regard to their potential therapeutic implications. Indeed, it has been shown that miRNAs may be efficiently long-term inhibited by antisense technologies, which has generated growing expectation in the inhibition of specific miRNAs as a potential therapeutic option for certain cardiovascular diseases [30].

miRNAs expression was also investigated in type 2 diabetes mellitus; using miRNA arrays, reduced levels of miR-126, miR-15a, miR-29b, and miR-223 and elevated levels of miR-28-3p were found in type 2 diabetic patients. *In vitro, in vivo* and clinical studies have revealed the association between miRNAs and some processes of insulin production and release, as cellular membrane electrical excitability, insulin granule exocytosis and insulin synthesis in pancreatic beta cells [31]. Moreover, the endothelial cell–derived miR-126, one of the identified downregulated miRNAs in atherosclerotic disease, was found most consistently associated with type 2 diabetes [32]. Since miR-126 has been shown to play an important role in maintaining endothelial cell homeostasis and vascular integrity [33,34], it has been suggested that this unique plasma miRNA might become a valuable tool to predict micro- and macro-vascular complications of diabetes. The analysis of circulating miRNA might be a good source of diagnostic and prognostic biomarkers also in metabolic diseases. To this end, longitudinal clinical studies with large sample size and with standardized system for the analysis of miRNA should be conducted to evaluate the clinical value of these nucleotides as biomarkers for predicting progression of metabolic diseases.

The presence of endogenous miRNAs in microparticles makes circulating miRNAs remarkably stable in the bloodstream, so they may be identified and measured in the circulation [12]; several techniques are available for quantifying circulating miRNAs, such as quantitative real-time Polimerase Chain Reaction (qRT-PCR) [35], Northern blotting [36], bead-based flow Cytometry [37], Microarray [38] or Deep sequencing [39]. However, of these assay, qRT-PCR seems superior because of its high sensitivity, specificity and reproducibility; moreover qRT-PCR requires less amount of RNA sample, usually more than 1 μg, but the number of miRNAs possible to analyze and RNA quantity may represent limitations for this assay [35]. Conversely, Deep sequencing technology has recently emerged as an attractive approach for miRNA analysis; in some cases, this technique showed most specificity and sensitivity compared to qRT-PCR and Microarray, also allowing identification of novel miRNA isoforms [40]. It is well known that a single gene may, in turn, be regulated by multiple miRNAs, therefore, given the large number of miRNAs annotated in the human genome, 30% to 80% of human genes are predicted to be influenced by miRNAs. Moreover, a single miRNA influences the expression of hundreds of unique miRNAs and aberrant miRNA expression may affect a multitude of transcripts and may profoundly influence disease-related signaling pathways. This situation generates a complex network so the analysis of miRNA panels is consequently more efficient in studies of diseases than the analysis of a single miRNA [41].

The current methods used for miRNAs detection usually require high costs and this aspect exerts limits on their use in daily clinical practice; in this way, research should try to overcome this limiting factor by developing less expensive detection techniques whereas spending review and cost-containment measures in health care represent a significant management problem [42].

However, given that current serological biomarkers, commonly employed in diagnosis and follow up of some diseases as cancer, acute myocardial infarction, atherosclerosis and diabetes seem to have lower specificity and sensitivity than miRNAs, it is plausible that circulating miRNAs detection may be included in future routine clinical examinations for management of these diseases. Unfortunately, in terms of sensitivity and specificity, to date there are only few small comparative studies between microRNAs and other conventional serological disease biomarkers commonly used in clinical practice.

Conclusion

In conclusion, the potential of circulating miRNAs as stable blood-based biomarkers for some diseases is promising and it is to be expected that combining multiple miRNAs into a miRNA profile may provide greater accuracy than can be expected from the assessment of a single miRNA. There are currently no circulating miRNAs that are validated as biomarkers for use in routine clinical practice; the lack of significative comparative studies between miRNAs and common disease biomarkers and high detection costs are the main limitations on the use of these nucleotides in daily clinical practice. In the next future, larger, comparative, long-term, randomized controlled trials must be undertaken, primarily in oncology, since the most significant results were obtained in this field. Particularly, new low-cost and wide availability assays to detect cancer biomarkers with high sensitivity and specificity need to be developed to improve screening protocols, early diagnosis, staging and follow up in neoplastic patients and to provide information on chemoresistance and the risk of relapses.

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