CIRCULATING MICRORNAS AS MEDIATORS IN CELL-TO-CELL COMMUNICATION

Christian Bär,[1] Thomas Thum,[1,2] David de Gonzalo-Calvo,[1,3,4,5,*]

[1] Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hannover, Germany.

[2] National Heart and Lung Institute, Imperial College London, U.K.

[3] *Institute* of *Biomedical Research* of *Barcelona* (IIBB) - Spanish National Research Council (CSIC), Barcelona, Spain.

[4] CIBERCV, Institute of Health Carlos III, Madrid, Spain.

[5] Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain.

Corresponding Author:

David de Gonzalo-Calvo, Ph.D.

Institute of *Biomedical Research* of *Barcelona* (IIBB) - Spanish National Research Council (CSIC)

Hospital de la Santa Creu i Sant Pau. C/ Sant Antoni Maria Claret 167, Pavelló del Convent, 08025 Barcelona, Spain

Tel: +34935565903, Fax: +34935565559

E-mail: david.degonzalo@gmail.com

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ABSTRACT

The number of publications that have evaluated the clinical application of circulating microRNAs as biomarker has exponentially grown in the last years. Paradoxically, the biology of these small non-coding RNAs in the circulation is far from being completely understood. Previous evidence suggests that circulating microRNAs may function as hormone-like mediators. Nevertheless, there are still fundamental gaps that need to be addressed to elucidate the precise role of circulating microRNAs as intercellular mediators including their true biological significance in homeostasis and disease of cell types other than their origin. Further efforts are required to determine their precise role in intercellular communication.

Running head: Circulating miRNAs and cell-to-cell communication.

Keywords: Biological transport; Cell-to-cell communication; Circulating microRNA; Endocrine genetic signal.

Since their discover in the blood circulation in 2008 [1], microRNAs (miRNAs) have emerged as a subclass of the non-coding RNA (ncRNA) superfamily that has gained most attention in biomarker development. Due to their biochemical properties, the

number of publications that have explored the clinical application of circulating miRNAs as diagnostic and prognostic indicators of cardiovascular disease has exponentially grown in the last decade [2]. Surprisingly, despite the enormous research and economic interest in miRNAs, the biology of these small ncRNAs in the circulation is far from being completely understood.

In 2007, Valadi and colleagues [3] proposed for the first time extracellular miRNAs as a novel paracrine mechanism for intercellular communication. Accordingly, extracellular miRNAs secreted by donor cells could be delivered into recipient cells where they function as endogenous miRNAs leading to altered gene expression. In just a few years thereafter, a growing body of data provided evidence for this hypothesis. *In vitro* studies suggest that the secretion of miRNAs is an active, controlled and specific process [4], that is presented in most, if not all, cell types. Different investigations have proposed miRNAs as intercellular communicators between cardiovascular cells, especially at the paracrine level. For instance, endothelial cell-derived apoptotic bodies generated during atherosclerosis contain miR-126 which acts as alarm signals to recipient vascular cells by inducing vascular protection [5]. In response to atheroprotective stimuli, endothelial cells secrete extracellular vesicles enriched in miR-143 and miR-145 both controlling target gene expression of co-cultured smooth muscle cells (SMC) [6]. Bang and colleagues [7] identified cardiac fibroblast-derived miR-21-3p as exosome-specific paracrine signaling mediator of cardiomyocyte hypertrophy.

In addition to these findings, recent published *in vivo* studies also suggest a "hormone-like behavior" of circulating miRNAs, especially in the context of cancer and metabolism. Focusing on the latter, Thomou and colleagues [8] demonstrated that circulating exosomal miRNAs participates in cell-cell crosstalk between adipose and liver tissue. Adipose-derived miRNAs enter the circulation and act as metabolic regulators in

target tissues by altering mRNA expression and translation. Supporting the biological significance of circulating miRNAs as adipokines, Ying and colleagues [9] showed that adipose tissue macrophages secrete miRNA-containing exosomes which can function as endocrine signals in the regulation of the overall glucose homeostasis.

Concerning the cardiovascular system, the data published thus far is limited. Hence, a robust causal link between circulating miRNAs and cardiovascular physiology and pathology is still lacking. The association between non-random circulating miRNA signatures with a wide array of cardiovascular conditions observed in patient-based studies suggests that circulating miRNAs are actively and selectively released from cells in response to stress or injury [10]. Therefore, it is entirely conceivable that circulating miRNAs can function as signaling molecules in cardiovascular health and disease. This concept is supported by *in vivo* studies demonstrating that the systemic delivery of miRNAs can induce biological responses in target cells and tissues [11]. Among the most convincing evidence that circulating miRNAs can act as hormone-like effectors in the cardiovascular system comes from Shan and colleagues [12], who reported circulating miR-223 as a novel endocrine genetic signal between blood cells, such as leukocytes and platelets, and vascular cells. Using both *in vitro* and *in vivo* approaches, the authors reported that blood cell–secreted miR-223 in serum enters into vascular SMC, which do not express miR-223, and regulates their proliferation, migration and apoptosis. Under atherosclerotic conditions, the secretion of miR-223 is increased which protects against atherogenesis. In a similar study, it has been described that blood cell–released miR-223 can also enter into endothelial cells and regulate the expression of its target genes [13]. Interestingly, this mechanism seems to be implicated in the vascular injury from Kawasaki disease.

Despite the promising findings, there are fundamental gaps that need to be addressed to elucidate the precise role of circulating miRNAs as intercellular mediators including their true biological significance in homeostasis and disease of cell types other than their origin. In this regard, different authors proposed that the concentration of miRNAs in plasma or serum is too low (from pM to fM) to induce biological or pathological responses in their target cells and/or tissues [14]. Nonetheless, in this discussion the mechanisms of action of miRNAs should be taken into account. Several miRNAs may regulate the expression of the same gene. In contrast, different miRNAs (or just a single miRNA) may target multiple genes in the same molecular pathway. Estimations need to consider the concentrations of all delivered miRNAs that can regulate a given molecular pathway in the recipient cell. Assuming a 1:1 stoichiometry between miRNAs and their mRNA targets may be an over-simplification. Since the bioinformatics tools available to explore this hypothesis have strong limitations [15], currently, this analysis is virtually impossible. Additionally, a model for the selective uptake of circulating miRNA allowing sufficient intracellular accumulation to repress target mRNA expression should not be completely discarded. Importantly, the cellular machinery involved in the packaging of miRNAs in membrane vesicles or in protein/lipoprotein complexes and the mechanism of selection and secretion of circulating miRNAs have not been fully revealed. In fact, the cellular origin of many circulating miRNAs has been matter of debate. Platelets have been identified as a major source of circulating miRNAs [16]. However, different pieces of evidence suggest that the circulating miRNA profile is the reflection of the secretory pattern of multiple hematopoietic and non-hematopoietic cells [17]. Target tissue specificity of miRNA carriers, the delivery pathways of circulating miRNAs and how they mediate their biological effect on the recipient cell are also barely understood. Indeed, in addition to the expected presence of mature single-

strand miRNAs in extracellular vesicles, precursor miRNAs (pre-miRNAs) and the machinery to process these pre-miRNAs into mature miRNAs has also been described in exosomes [18].

The technical limitations are a key aspect that deserves a careful discussion. Although different initiatives are working on the standardization of the methodology [19], there are considerable hurdles that should be overcome especially in the isolation of miRNA carriers and the quantification of circulating miRNA expression levels [20, 21]. The use of artificial delivery systems and supraphysiological concentrations of exogenously applied miRNAs in *in vitro* and *in vivo* experiments should also be considered in the interpretation of the experimental data [14]. The cellular responses observed in these artificial conditions do not necessarily be biologically relevant. Furthermore, the effect of miRNA carrier components on endogenous miRNA expression is a confounding factor that should be appropriately addressed (e.g. knockout models) in case the recipient cell is capable of expressing the miRNA of interest.

In conclusion, circulating miRNAs may function as active hormone-like mediators. Understanding of the biology of circulating miRNAs is still at an early stage; and therefore, further efforts are required to determine their precise role in intercellular communication. The characterization of circulating miRNAs as endocrine genetic signals will provide exciting opportunities, not only for a better understanding of cardiovascular pathologies, but also for biomarker development and for the design of novel therapeutic approaches.

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