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Microparticles and MicroRNAs of Endothelial Progenitor Cells Ameliorate AKI

Markus Bitzer[1], **Iddo Z. Ben-Dov**[2], and **Thomas Thum**[3,4]

[1]University of Michigan, Department of Internal Medicine, Nephrology, Ann Arbor, MI

[2]Rockefeller University, Howard Hughes Medical Institute, Laboratory of RNA Molecular Biology, New York, NY

[3]Institute for Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hannover Germany

[4]Centre for Clinical and Basic Research, IRCCS San Raffaele, Rome, Italy

Abstract

Horizontal information transfer between cells via microparticles is a newly identified communication system. MicroRNAs regulate gene expression and are detected in microparticles. The article by Cantaluppi et al. suggests that microparticles derived from circulating angiogenic cells ("endothelial progenitor cells", EPC) harbor endothelial-protective miRNAs such as miR-126 and that delivery of EPC-derived microparticles during acute kidney ischemia-reperfusion in rats ameliorates kidney dysfunction and damage. We highlight the importance, future impact and limitations of this study.

Most if not all cell types of diverse organisms release membranous nano-sized vesicles that harbor RNA, proteins and lipids. These microparticles (MP) include microvesicles (100 nm to 1 μm) generated by surface shedding of plasma membrane containing cytoplasm; and exosomes (40 to 100 nm) originating from the membrane of endosomal multivesicular bodies (MVB) and released into the extracellular environment during exocytosis upon fusion with the plasma membrane. The function of these extracellular vesicles is just beginning to become apparent. They play a role in cell–cell communication, and may have pathologic functions in immune processes and cancer progression (Figure).

In kidney research, urinary MPs are being actively investigated as biomarkers and mediators of extracellular communication between renal epithelial cells [1]. Currently, only a few studies have examined the impact of MPs derived from extrarenal cells on kidney injury. MPs originating from mesenchymal stem and endothelial progenitor cells were found to convey signals that ameliorate tubular injury. For endothelial cell-derived MPs, horizontal transfer of mRNA and activation of an angiogenic program in the recipient endothelial cells has been suggested as the underlying mechanism [2]. MicroRNAs (miRNAs), small regulatory RNA molecules, are actively secreted packaged in MPs and/or bound to proteins from different cell types including mast cells and embryonic stem cells [3]. The manuscript by Cantallupi et al. [4] shows that MPs from endothelial progenitor cells (EPCs) isolated from PBMCs of healthy human donors protect against acute and long-term consequences of

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ischemia-reperfusion injury (IRI) in rats, if injected in the tail vein immediately after ischemic injury. MPs derived from fibroblasts or from EPCs treated with Dicer siRNA or miR-126 and miR-296 inhibitors or preincubated with RNase, failed to ameliorate IRI. In addition, hypoxia-induced uptake of MPs diminishes apoptosis and promotes pro-angiogenic and anti-apoptotic gene expression in cultured primary tubular endothelial and epithelial cells, in a manner at least partially dependent on miR-126/miR-296.

These findings support previous work by the authors and other groups on the role of progenitor cells in kidney injury but also point out gaps in our understanding of MPs and their cargo: What is the difference between vesicles that ameliorate injury (MPs derived from EPCs) and those that have less or no effect (MPs from fibroblasts)? In this respect it needs to be emphasized that the term “EPC” is indeed controversial as it may include multiple cell types with different functions. EPCs circulate in the bloodstream and contribute mainly by paracrine actions to formation of new blood vessels, endothelial repair and vascular homeostasis [5]. Of clinical relevance, impairment of EPC is related to endothelial dysfunction and adverse clinical outcome [6]. EPCs are obtained by an adhesion-related isolation method and are defined by expression of the endothelial lineage markers such as CD31, KDR (VEGFR-2), VE-cadherin, and the von Willebrand factor (vWF). EPCs also show certain endothelial properties such as migration towards pro-angiogenic factors. However, these early EPCs are also referred to as circulating angiogenic cells (CAC), monocytic EPC, early outgrowth EPC or angiogenic progenitor cells (APC) [5]. The cell type used by many groups and investigated by the Camussi group can also be described by the term “circulating angiogenic cells”. Future research will have to determine if the findings of Camussi’s group with regard to the observed beneficial effects and miRNA content are generally comparable between different “EPC” subtypes identified so far.

The current paper attributes the protective effect to miR-126 and miR-296, which are detected in the microvesicles by RT-PCR. Mouse knock-out of miR-126 was found to be the cause of a severe vascular phenotype initially ascribed to its host gene, *Egfl7* [7]. Similar to its host gene, miR-126 is primarily expressed in endothelium. In a large small-RNA sequencing database miR-126 composed up to 10–15% of the total miRNA content in tissues with a dominant endothelial component (angiosarcoma, microdissected glomeruli, heart), with levels ~3–5% noted in CD34+ lymphocytes, PBMCs and fetal lung (personal communication, Thomas Tuschl). As EPC frequently can take up platelets and thus also their genetic content the real source of certain miRNAs detected in EPCs remains to be determined. MiR-126 and many of its targets are highly expressed in endothelial cells, and both, miR-126 and miR-296 have been implicated in angiogenesis [8] (however, expression in the tissue database was not nearly as high for miR-296, and no enrichment was noted in endothelium containing samples). The notion that miRNAs are also transferred to other cell types urges us now to identify also cell-type specific targets of transferred miRNAs, especially if they are normally not expressed in this cell type, which is the case for tubular epithelial cells and miR-126 (Ben-Dov IZ, unpublished). Thus, the targets of miR-126 in diverse kidney cells remain to be determined.

MiRNAs might be enriched in MPs, and the machinery required for miRNA function has recently been linked to MVBs. MiRNAs require the RNA-induced silencing complex

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(RISC) for downregulation of expression of target genes, but formation and turnover of the RISC complex itself is tightly regulated and requires endosomal membranes. MVB associate with RISC and GW-bodies and blocking MVB formation results in impaired silencing of mRNA by miRNAs indicating that active RISC is physically and functionally coupled to MVBs [9]. It remains to be determined whether this association results in enrichment of RISC-associated small RNAs in exosomes, which originate from MVBs and in regulation of miRNA content in MPs in physiology and disease.

Notwithstanding the above discussion, it cannot be ruled out that the cargo RNA mediator of the protective effect of MPs is not the miRNA but an indirect mRNA target. For this to happen, the putative transcript would have to be upregulated by miR-126; i.e. miR-126 targets a repressor of this mRNA. In fact, the latter mechanism may be favored based on stoichiometric considerations. Assuming the total take-up of MPs by the cell amounts to 1:1000 of its volume (likely an overestimate), the delivered miRNA, even if enriched in the vesicle, is much diluted compared to its concentration in the parent cell. Since miRNA perform their function at 1:1 basis with their targets (and must compete with ‘native’ miRNA on RISC-loading), significant regulation of a target gene is implausible in most cases. On the other hand, a newly introduced mRNA is more likely to cause a switch as it can be used to generate many protein molecules, departing from the 1:1 relationship. Additionally, mRNAs are also more susceptible to ribonuclease digestion; consistent with the findings by the authors that RNase treatment abolishes the effect of MPs.

The findings reported by Cantaluppi et al. suggest that MP from unchallenged endothelial progenitor cells protect from IRI damage. It can be speculated whether circulating MPs constitute an additional physiological mechanism to counter endothelial damage that may be altered in disease states. Furthermore, the authors show that dye-labeled MPs were detected not only in endothelial but also in tubular epithelial cells 2h after tail vein injection and that MPs have measurable effects in cultured tubular epithelial cells. As MPs appear to be too large to enter the tubular lumen through the glomerular filtration barrier to reach the tubular epithelial cells via the urinary space, MPs would need to migrate from the blood through the basal membrane to the tubular epithelium.

Current knowledge supports further exploration of the complex interplay between the microRNA machinery and endosomal vesicles, and the mechanisms of cargo loading into microvesicles and exosomes, recognition of target cells by MPs and alteration of gene and protein expression in target cells by cargo of MPs (Figure). Better understanding of the biology of MPs and their content is important before genetically altered vesicles can be considered as potential novel therapeutic carriers.

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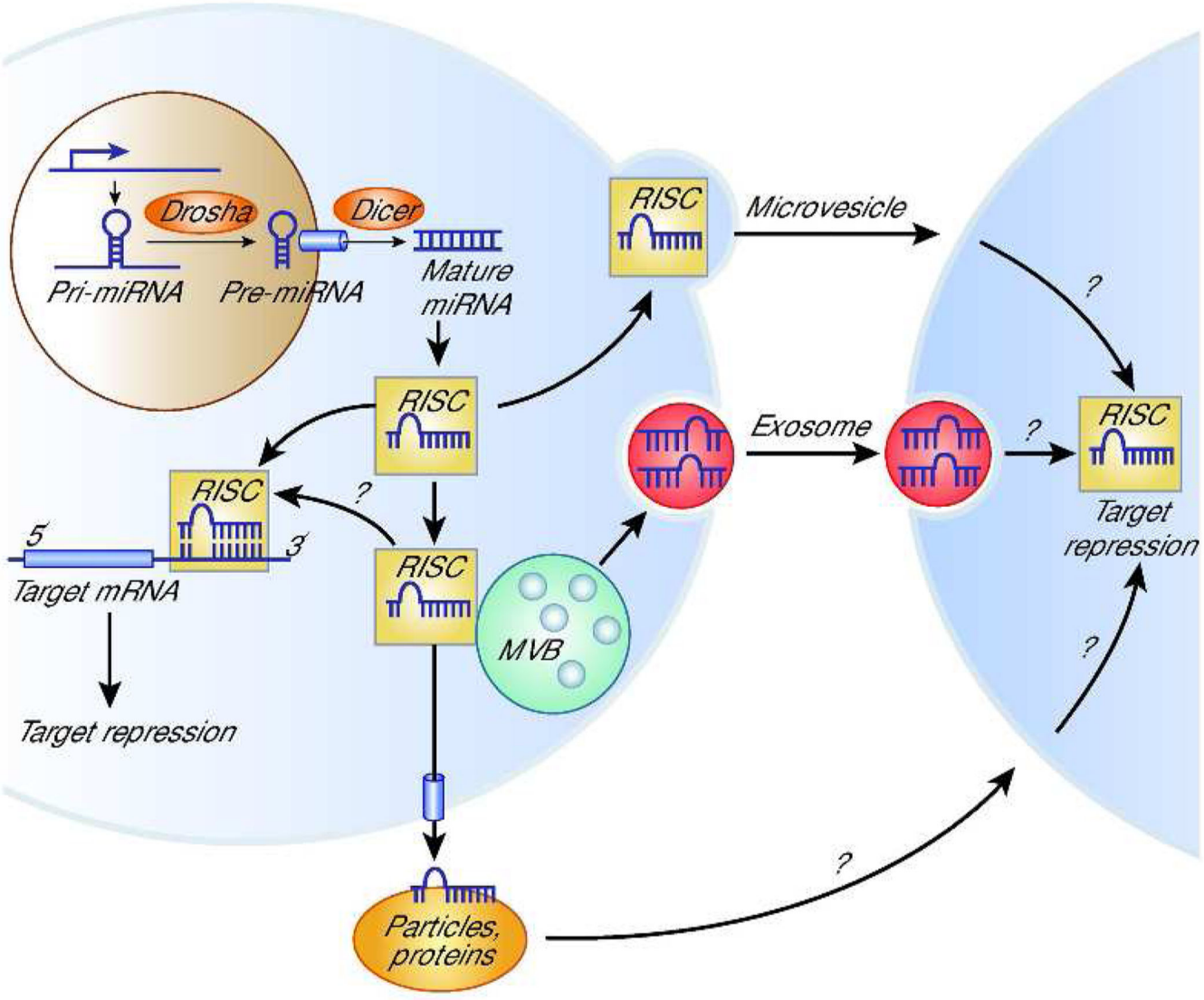

Figure.

MicroRNA biogenesis and transport. MicroRNAs are transcribed from microRNA genes that are similar to protein-coding genes into pri-miRNAs (several hundreds nucleotides long, harboring 5' cap and 3' poly-A tail), further processed into pre-microRNAs (~70 nucleotides long with a characteristic hairpin loop) by the RNase III enzyme Drosha in the nucleus, shuttled into the cytoplasm through Exportin 5, where the RNase III enzyme Dicer generates mature microRNAs bound to its star or passenger strand. Mature microRNAs are then incorporated into the RNA-induced silencing complex (RISC) where target mRNAs are either degraded or translational repressed through partial sequence complementation. In the extracellular space microRNAs have been detected in microvesicles and exosomes, and bound to extracellular particles and proteins. The RISC complex has been found to be associated with the membranes of MVBs derived from the endosome and in micovesicles; it

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is likely also in exosomes. The mechanisms underlying uptake of microRNAs into vesicles and the release from the vesicles in target cells is largely unknown.