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Review Article

Extracellular vesicle microRNA transfer in cardiovascular disease

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ABSTRACT

microRNAs (miRNAs) are a class of small regulatory RNAs that decrease protein translation to fine-tune cellular function. Recently, miRNAs were found to transfer from a donor cell into a recipient cell via exosomes and microparticles. These microvesicles are found in blood, urine, saliva, and other fluid compartments. miRNAs are delivered with intact functionality and have been repeatedly shown to regulate protein expression in recipient cells in a paracrine fashion. Thus, transported miRNAs are a new class of cell-to-cell regulatory species. Exosomal miRNA transfer is now being reported in cardiovascular systems and disease. In the blood vessels, this transfer modulates atherosclerosis and angiogenesis. In the heart, it modulates heart failure, myocardial infarction, and response to ischemic preconditioning. This review describes our current understanding of extracellular vesicle miRNA transfer, demonstrating the roles of miR-126, miR-146a, miR-143, and other miRNAs being shuttled from endothelial cells, stem cells, fibroblasts and others into myocytes, endothelial cells, and smooth muscle cells to activate cellular changes and modulate disease phenotypes.

1. Introduction

1.1. A brief primer on microRNAs

microRNAs (miRNAs) are 18- to 25-nucleotide (nt) small RNA species that serve as master regulators of translation. Over the last decade or so, an explosion of new discoveries has identified important roles for miRNAs in health and disease. miRNA expression levels change in response to cell stressors modulating entire regulatory pathways and fundamentally altering cellular biology [1]. miRNAs have a clearly established role in cardiovascular function and disease [2–5].

The complex maturation and activity sequence for miRNAs has been generally solved. miRNAs are typically transcribed as longer pri-miRNAs containing one or more pre-miRNA hairpin structures of ~80 nt length. Within the nucleus, these pri-miRNAs are cleaved by the class 2 ribonuclease III Drosha to form miRNA hairpins. These pre-miRNA hairpins are exported into the cytoplasm where they are further cleaved by the endoribonuclease Dicer into two separate miRNAs commonly referred to as the mature and passenger (or * strand) miRNA. The mature miRNA is preferentially loaded into the RNA-induced silencing complex (RISC) containing multiple proteins, while the passenger miRNA is most frequently (but not always) degraded [6]. In quiescent tissues, most of the RISC complex is a low-molecular-weight structure with reduced regulatory activity, while stressed or replicating cells have more active high-molecular-weight RISC complexes that are bound to mRNAs [7]. miRNAs have a 6-nt seed-match site, from position 2 to 7 in their 5′ end that is complementary to regions of mRNAs (most often in the 3′ UTR). The binding of a miRNA to an mRNA within the RISC complex will either result in mRNA degradation or translation suppression, resulting in lower protein levels [8]. Due to the relatively short complementary sequence and the functional suppression despite imperfect nucleotide matches, each miRNA can regulate hundreds of different proteins. Identifying all of the regulatory functions of each miRNA is underway but is still at an early state of discovery [9].

In addition to determining their mechanism of action, a fair amount of research has attempted to catalog all of the miRNAs and determine their localization. The desire to organize the known miRNAs has led to a central repository of miRNAs, miRBase.org, and a common naming convention [10]. The latest release at miRBase.org (v21) listed 2578 human miRNAs, but recent publications suggest that there are many more miRNAs to be discovered, particularly those that are lineage specific [11]. miRNAs can be transcribed from throughout the genome and are found with greater frequency within introns or in bicistronic/polycistronic clusters. Rather than the entirety of all known miRNAs being active within a cell, it is believed only a few hundred miRNAs are present in any cell at levels high enough to regulate protein synthesis [12]. In fact, miRNA expression varies widely by cell type and cell maturation. In many cases, the cellular origin of a miRNA is unknown, as most characterization has been at the tissue level, with fewer studies performed at the cellular level [13–16].

To keep this class of RNAs well organized, a specific naming convention has been developed. The first three characters of the miRNA refer to the species ('hsa' for humans, 'mmu' for mice), the next three characters are 'miR' (or 'let' for this special family), and the next part is a number for the specific miRNA, followed by the part of the hairpin '-5p' or '-3p'

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from which the miRNA came. Either of the -5p or -3p strands can be the mature or passenger (*) strand with roughly equal frequency. Often, in describing miRNAs, the species name is dropped and the -5p or 3p designation is dropped when referring to the mature (dominant) strand as is done in this review.

1.2. A brief primer on extracellular vesicles

Extracellular vesicles (EVs) represent several classes of small membrane-enclosed vesicles that are generated by cells. They are generally known to signal and be involved in cell-to-cell communication. EVs are made by a variety of cell types, some that appear to be constitutively released to maintain the cellular homeostasis and others that increase their release based on stimuli. EVs are found in blood, urine, cerebral spinal fluid, and other biological fluids [17].

Research into EV biology has been complicated by a large number of definitions and overlapping nomenclature of a range of vesicles. EVs include exosomes, ectosomes, microvesicles, microparticles, apoptotic bodies, budding vesicles, shedding microvesicles, and other EV subsets [18]. These terms have been used interchangeably, particularly the term "exosome" that has become a catch-all despite stricter definitions. True exosomes are ~60 nm (30–100 nm), double-membraned vesicles that are formed by the recruitment of the protein Alix to the surface of early endosomes in the endosomal multivesicular compartments (Fig. 1). These exosomes become secreted when fused with the plasma membrane. Exosomes contain a set of specific proteins including the surface tetraspanins (CD9, CD63, CD81, etc.). Exosome release is dependent upon Rab27a and Rab27b that dock exosomes to the plasma membrane [19]. Another class of EV is the microparticle. It is derived as a shedded vesicle directly through plasma membrane blebbing. These vesicles are not formed in the endosomal compartments. They are usually larger than exosomes (300 nm to 1 μm) and do not contain tetraspanins.

One reason that the literature concerning EV is so muddied is that there is no agreed upon mechanism to isolate exosomes or microvesicles from plasma, serum, or cell culture media [17]. Salting out kits, ultracentrifugation, flow cytometry, column purification, magnetic bead purification, and others have all been used each resulting in different yields and specificities. Thus, almost all research reports studying EVs will, in actuality, contain vesicles from multiple classes, despite the use of terminology that implies a more pure preparation such as 'exosome'. For the purposes of this review, all manner of microparticles, exosomes, and other vesicles captured by whatever isolation method that was used by the investigators are being referred to as EVs, unless specifically stated otherwise and with evidence of a robust and validated isolation method.

1.3. miRNAs as EV biomarkers

When miRNAs were first described as being present in biological fluids (blood, urine, etc.) they were proposed to be useful biomarkers of disease [20]. Levels of either free miRNAs bound only to a protein complex such as Argonaute (Ago) or miRNAs within EVs have been assayed for correlation to a wide variety of diseases. In the oncology literature, hundreds of studies have been performed generating a variety of outcomes of both positive and negative associations of miRNAs, much of which has been questioned on methodologic grounds [21–23]. miRNAs as blood-based biomarkers have also been investigated in a huge variety of nonneoplastic diseases ranging from Alzheimer's disease to zinc depletion. Here again, there appears to be little specificity of a miRNA expression change for a particular disease, with six common miRNAs being implicated as specific biomarkers in nine or more different diseases [24].

Among cardiovascular diseases, a specific set of miRNAs are reproducible and robust biomarkers of myocardial infarction (MI) [24,25].

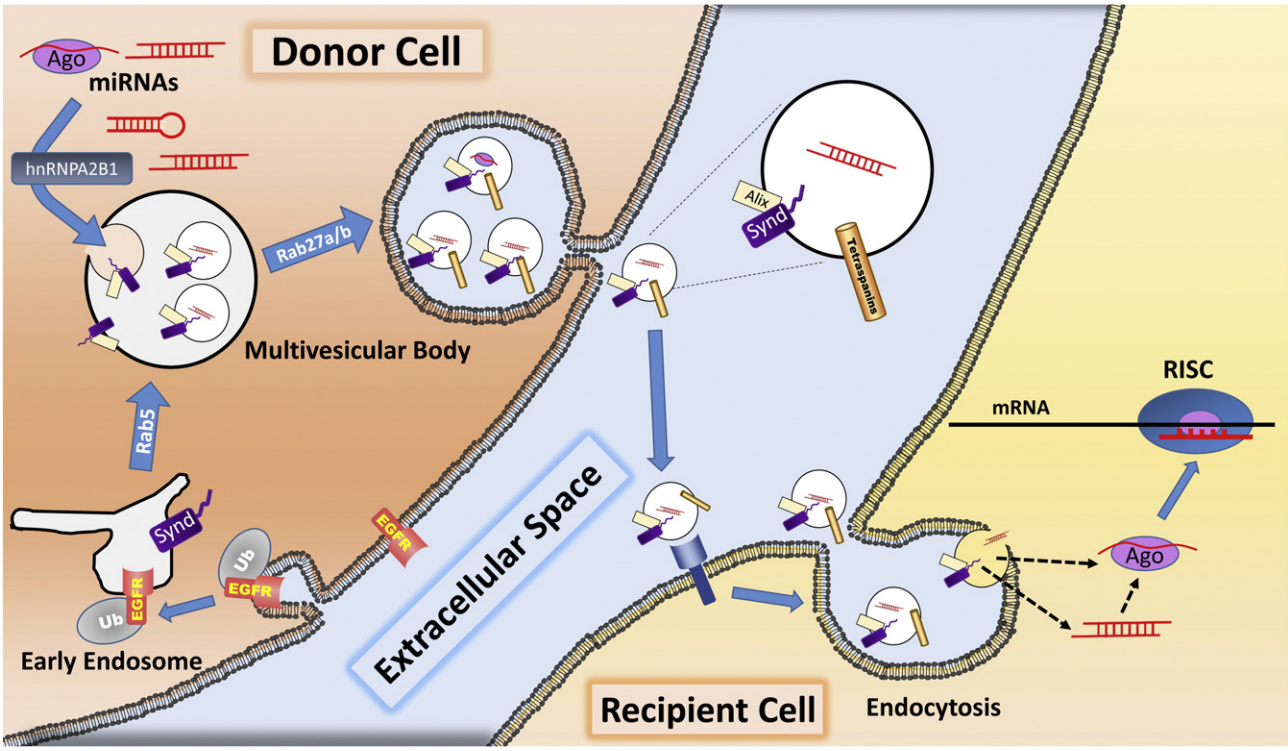

Fig. 1. General mechanism of miRNA-containing exosome formation, release, and uptake. A surface receptor such as the EGF receptor EGFR is ubiquitinated (Ub). This results in movement of the surface membrane and proteins into an early endosome with the recruitment of Syndecan (Synd). Alix binds to Synd and moves in a Rab5-dependent pathway to form an endosome/multivesicular body. Multiple exosomes are pinched off from the endosome membrane capturing cell contents including miRNAs. hnRNPA2B1 may help preferentially package certain miRNAs. These endosomes then require Rab27a and Rab27b to dock to the cell membrane and release the tetraspanin (CD9, CD63, CD81, etc.) positive exosomes into the extracellular space (including serum and other biological fluids). Circulating exosomes are recognized and bound by a variety of receptors on recipient cells. The receiving cell then performs endocytosis, receiving the exosome cargo. The mature or Ago-bound miRNA is ultimately loaded into a RISC complex to repress mRNA translation.