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Cell-to-cell miRNA transfer: From body homeostasis to therapy

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ABSTRACT

The role of non-protein coding RNAs (ncRNAs), microRNAs (miRNAs) in particular, as fine-tuners of both pathological and physiological processes is no longer a matter of debate. With the recent discovery of miRNAs in a wide variety of body fluids and considering them as tools employed in horizontal gene transfer between cells, a new horizon opens in the field of diagnosis and therapeutics. Circulating miRNAs not only enable the communication among cells, but also provide insight into the pathological and physiological state of the originating cells. In this review we summarize the recent advances made in this field, arguing for compelling translation of miRNAs into clinical practice. Moreover, we provide overview of their characteristics and how they impact the evolution of tumor microenvironment and cell-to-cell communication, advancing the idea that miRNAs may function as hormones.

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1. Introduction

Victor Ambros's and Garry Ruvkun's discovery of miRNAs revolutionized research and changed the scientific world's perspective towards the traditional dogma: DNA → RNA → Protein. Most of the inquiries have been conducted in the cancer field, considering that miRNAs were first linked to this malignancy a decade ago (Calin et al., 2002). While their reputation as master regulators of almost all biological processes spread rapidly throughout the medical world, it has triggered the interest of scientists working in various fields and as our knowledge about diseases continuously expands, new roles of these small non-coding RNAs have been revealed.

Tumors are no longer being regarded as a collection of relatively homogeneous cancer cells, but rather as a complex assemble of distinct cell types (Hanahan & Weinberg, 2011) in which cell-to-cell communication is essential for the regulation of proliferation, angiogenesis and metastasis (Hu & Polyak, 2008). Furthermore, if one is to look at cancer through the lens of evolution and ecology, tumor microenvironment can be considered a dynamic ecosystem obeying Darwin's theory for the selection of the "fittest" cancer cells (Hede, 2009). In this context, horizontal gene transfer (HGT), a mechanism initially described in bacteria for passing of genetic material between organisms, that provides

Abbreviations: miRNA, microRNA; HGT, horizontal gene transfer; HDL, high-density lipoprotein; MVB, multivesicular body; nSMase2, sphingomyelinase-2; DC, dendritic cells.

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selective advantage in particular environments, emerges as extremely relevant, and various recent studies have advanced the idea that it may occur in multicellular organisms as well (Ratajczak et al., 2006; Valadi et al., 2007; Ahmed & Xiang, 2011). HGT through secreted miRNAs is a newly introduced concept aiding the elucidation of cell-to-cell interactions and the mechanisms of co-evolution of tumor cells and their microenvironment. Nevertheless, it is mandatory to point out that here we refer to HGT occurring without genomic integration. Moreover, analyzing miRNAs from this angle grants the means for regarding them as the last addition to the expanding world of hormones.

In this review, we will describe the known characteristics of secreted miRNAs and focus on their impact on the evolution of tumor microenvironment and cell-to-cell communication, highlighting the implications of secreted miRNAs in therapeutics and arguing for their relationship to hormones.

2. What are microRNAs?

The role of non-protein coding RNAs (ncRNAs) as fine-tuners of both pathological and physiological processes is no longer a subject of debate. Findings over the past several years have linked this class of nucleic acids, once considered 'background noise', with a large panel of biological processes, such as homeostasis, development and carcinogenesis. MiRNAs are the members of this class that have seized all of the attention since their documented involvement in human diseases.

These small, non-coding RNAs commonly found intracellulary, are 20–23 nucleotides long and expressed in a tissue and developmental specific manner (Ambros, 2003). They can arise from intergenic or intragenic (both exonic and intronic) genomic regions and are transcribed as long primary transcripts (pri-miR), which fold back to form double stranded hairpin structures. Primary transcripts are subjected to sequential processing: first the precursor molecules (pre-miR), 80–120 nucleotides long, are produced in the nucleus by type III endonuclease DROSHA, followed by their export to the cytoplasm mediated by EXPORTIN5, where they are processed by another type III endonuclease, DICER into the short "active" molecules (Kim, 2005).

Commonly, miRNAs negatively regulate gene expression via either mRNA cleavage or translation repression (He & Hannon, 2004), yet it was recently shown that they can upregulate the expression of their target genes as well (Vasudevan et al., 2007). Due to the ability of a single miRNA to target hundreds of mRNAs and their involvement in virtually all biological processes, aberrant miRNA expression is associated with the initiation of many diseases, including cancer.

3. Circulating microRNAs

The recent detection of miRNAs in body fluids (e.g. blood, saliva, serum, milk) has led researchers to assign them the intriguing role of gene regulator molecules, in addition to their obvious role as biomarkers (Mitchell et al., 2008; Hu et al., 2010; Huang et al., 2010).

The secretory mechanism remains yet unclear, but three different possibilities have been suggested:

i. Passive leakage from cells due to injury, chronic inflammation, apoptosis or necrosis, or from cells with short half-lives, such as platelets (Chen et al., 2008; Mitchell et al., 2008)
ii. Active secretion via cell-derived membrane vesicles (nanovesicles), including exosomes, shedding vesicles and apoptotic bodies (Valadi et al., 2007; Zernecke et al., 2009; Zhang et al., 2010)
iii. Active secretion by a protein–miRNA complex: studies have shown the association of miRNAs with both lipoproteins (e.g. high-density lipoprotein — HDL) and proteins (e.g. Ago2) (Arroyo et al., 2011; Turchinovich et al., 2011; Vickers et al., 2011).

Many studies have systematically shown the remarkable stability of secretory miRNAs, despite the austere conditions they are subjected to in both the blood stream (RNase digestion) and during handling (e.g. extreme temperatures and pH values) (Chen et al., 2008; Boeri et al., 2011).

4. Molecular mechanisms of microRNA transfer

4.1. Cell-derived membrane vesicle-mediated transfer

The first evidence of encapsulation of miRNAs into nanovesicles (erroneously called microvesicles, in view of their size) was reported by Valadi et al. (2007), who stated that mast cell exosomes containing RNA (mRNA and miRNA) from mouse, are transferred to both human and other murine cells. After this transferral, new murine proteins were found in the recipient cells, demonstrating that exosomal mRNA can be passed into other cells. Thus, it was established that the message delivered on from the donor cells to the neighboring cells via exosomes, does not simply mirror the transcriptional status of the donor cell.

Exosomes are secretory products of endosomal origin with a diameter of 30 to 100 nm (Thery et al., 2002; Simons & Raposo, 2009; Mathivanan et al., 2010). They arise when cell membrane proteins transfer to early endosomes by inward budding. Intraluminal vesicles then develop via invagination of the endosomal membrane, generating endosomal carrier vesicles or multivesicular bodies (MVBs). MVBs are key players in endolysosomal transport. Exosomes are stored as intraluminal vesicles in MVBs, further being either degraded by fusion of MVBs with lysosomes or released into the extracellular milieu by fusion of MVBs with the plasma membrane. The formation of exosomes is facilitated by endosomal sorting complexes required for transport (ESCRT) proteins, which are multiprotein complexes consisting of the vacuolar protein sorting family of proteins (Babst, 2005). An alternative pathway, independent of the ESCRT machinery has also been described, and it includes the ceramide and sphingolipid pathway, in which the enzyme sphingomyelinase-2 (nSMase2) is involved in mediation of exosomal release (Marsh & van Meer, 2008; Trajkovic et al., 2008). Exosomes are released by a wide spectrum of cell types (e.g. monocytes, B cells, T cells, mast cells, epithelial cells), however their secretion is constitutive and exacerbated in cancer cells, and it appears to be modulated by microenvironmental milieu, influenced for instance by growth factors, heat shock and stress conditions, pH variations and therapy (Parolini et al., 2009; Hedlund et al., 2011; Khan et al., 2011; Ciravolo et al., 2012).

Shedding vesicles share properties with classical vesicles, although they are more heterogeneous in shape and larger (up to 1000 nm) than exosomes (Cocucci et al., 2007; Cocucci et al., 2009; Mathivanan et al., 2010) (Fig. 1). They are released by cells, both at rest and upon stimulation, via outward budding and fission of the plasma membrane and thus can be molecularly different. The shedding vesicles of tumor cells and neutrophils are enriched with metalloproteinase as well as other proteolytic enzymes used for the digestion of the extracellular matrix, necessary for the progress of inflammation and tumor growth (Gasser et al., 2003; Mochizuki & Okada, 2007; Giusti et al., 2008). Analogous to exosomes, shedding vesicles are dispensed by a broad range of cells, usually mixed with exosomes (Cocucci et al., 2009) and the delivery of their content seems to be facilitated through receptor–ligand interaction.

Other studies followed to confirm and extend to the discovery by Valadi and colleagues. For example Hunter et al. (2008) identified and defined the profile of exosome-encapsulated miRNAs circulating in the plasma of healthy individuals. Additionally, they found that 37 miRNAs were expressed at significantly different levels between plasma nanovesicles and peripheral blood mononuclear cells, and that the majority of these miRNAs were predicted to regulate cellular differentiation of blood cells and metabolic pathways. Another study determined that nanovesicles originated from human bone marrow-derived mesenchymal stem