Published in final edited form as:

Biol Cell. 2015 March ; 107(3): 61–77. doi:10.1111/boc.201400081.

Immunomodulatory role of microRNAs transferred by extracellular vesicles

Lola Fernández-Messina^{1,2}, Cristina Gutiérrez-Vázquez², Eva Rivas-García¹, Francisco Sánchez-Madrid^{1,2,*}, and Hortensia de la Fuente²

¹Immunology Service. Hospital de la Princesa. Madrid, Spain

²Department of Vascular Biology and Inflammation. National Centre for Cardiovascular Research (CNIC). Madrid, Spain

Abstract

The immune system is composed of different cell types localized throughout the organism to sense and respond to pathological situations while maintaining homeostasis under physiological conditions. Intercellular communication between immune cells is essential to coordinate an effective immune response and involves both cell-contact dependent and independent processes that ensure the transfer of information between bystander and distant cells. There is a rapidly growing body of evidence on the pivotal role of extracellular vesicles (EVs) in cell communication and these structures are emerging as important mediators for immune modulation upon delivery of their molecular cargo. In the last decade, EVs have been shown to be efficient carriers of genetic information, including microRNAs, that can be transferred between cells and regulate gene expression and function on the recipient cell. Here we review the current knowledge of intercellular functional transfer of EV-delivered microRNAs and their putative role in immune regulation.

Keywords

Extracellular Vesicles (EVs); Exosomes; microRNA (miRNA); immune modulation; intercellular communication

Introduction

Intercellular communication is a key process in multicellular organisms, generally achieved through direct cell-cell contact or transfer of secreted molecules. Mechanisms of immune cell-cell communication include the release of soluble factors, e.g. hormones, cytokines or chemokines, and cell contact-dependent molecular transfer processes, such as trogocytosis, nanotubes or nibbling. The release of membrane vesicles constitutes a conserved mechanism to facilitate cell communication, both in prokaryotes and eukaryotes. In the last years, extracellular vesicles (EVs) have emerged as potent mediators for signalling, as they carry bioactive molecules that can modulate the function of recipient cells. However, the concept

*Correspondence and requests should be addressed to F.S.M. (fsmadrid@salud.madrid.org).

of EVs as carriers of genetic information in mammals is very recent. Indeed, the first evidence for the transfer of EVs enriched in mRNA (Ratajczak et al. 2006) and microRNA (miRNA) species (Valadi et al. 2007), capable of inducing changes in the recipient cell, came from studies published less than a decade ago. Once released from cells, EVs containing miRNAs (EV-miRNAs), are transported in blood or other body fluids, such as urine, saliva, cerebrospinal fluid or breast milk, suggesting a potential role in both local and systemic intercellular transfer of information. It is worthwhile mentioning that local processes, e.g. in the tumour microenvironment, are not always reflected systemically. Thus, whether EV-miRNAs have local and/or distant effects should be evaluated in each specific context. The transfer of miRNAs, regulating gene expression in neighbouring cells is a highly conserved mechanism of intercellular communication described in plants (Zhang et al. 2012), neurons (Lehmann et al. 2012), smooth muscle (Hergenreider et al. 2012) or immune cells (Mittelbrunn et al. 2011) among others. This process has been shown to be involved not only in physiological conditions, participating in cell adhesion, migration or differentiation but also in pathological processes, such as viral infections, cardiovascular diseases or cancer.

In order to efficiently sense and respond to pathogen challenges while avoiding autoimmunity or exacerbated inflammation, the immune system needs to be fine-tuned. Although both EVs and miRNAs have been shown to have important functions for immune modulation, the immunoregulatory role of EV-delivered miRNAs remains elusive. In this review we provide current understanding of immunoregulation mediated by EV-shuttled miRNAs and discuss future perspectives for their use as biomarkers and targeted gene therapy.

I Extracellular Vesicles and the Immune Response

In the last years the transfer of EVs has emerged as a novel mechanism of cell communication. Most cell types, both immune and non-immune cells, release EVs. Although there is no general consensus on their nomenclature, EVs can be classified according to their size, density and subcellular origin into i) *ectosomes*, budding directly from the plasma membrane, also known as *microparticles* or *microvesicles*; ii) *exosomes*, from endocytic origin and iii) *apoptotic bodies*. Very comprehensive reviews describe the biogenesis and trafficking of the various types of EVs in detail (Thery et al. 2002b, Raposo and Stoorvogel 2013), so these issues will not be further discussed. Nonetheless it is important to point out that the confusing nomenclature used to designate EVs and the different methods used for their isolation render the data available in the literature often confusing and difficult to interpret (Gould and Raposo 2013). Nowadays, technical standardization for isolation and analysis of EVs is a priority in the field (Witwer et al. 2013). For the sake of a more comprehensive presentation of the data in this review, the general term EV will be used unless authors refer to a specific kind of EV.

EVs contain lipids, proteins and nucleic acids derived from the releasing cell (Thery et al. 2002b, Maule and Weber 2010) and have been shown to be involved in many physiological and pathological processes (Simons and Raposo 2009). The mechanisms underlying the sorting of molecules into exosomes are far from being understood, however high throughput

proteomic and deep-sequencing analyses of EVs show that their content is not random (Villarroya-Beltri et al. 2014). Post-translational modifications, such as addition of ubiquitin-like modifiers, have been detected in exosomal proteins and it has been proposed that these modifications may target proteins for EV sorting and release (Moreno-Gonzalo et al. 2014).

Concerning miRNAs, exosomes contain specific repertoires that differ from those of their parental cells as described in T lymphocytes and dendritic cells (Skog et al. 2008, Mittelbrunn et al. 2011, Nolte-'t Hoen et al. 2012). Specific miRNA signatures of exosomes as compared to parental cells such as miR-451 and miR-503 in mast cells have also been described (Ekstrom et al. 2012). Interestingly, miRNA repertoires of exosomes from different cellular origin shared a higher similarity than comparing to their corresponding parental cell, suggesting the presence of sorting mechanisms for EV loading (Mittelbrunn et al. 2011). Recently, it has been reported that miRNAs contain specific motifs that drive their inclusion into exosomes. The binding of the heterogeneous nuclear ribonucleoprotein A2B1 (hnRNP A2B1) to exosomal miRNAs through the recognition of these sorting motifs has been reported to control their loading into exosomes (Villarroya-Beltri et al. 2013). A recent study also proposes that miRNAs from either exosomal or cellular origin can be determined according to their nontemplated nucleotide additions (NTA) (Koppers-Lalic et al. 2014). The authors propose that isomiRs with 3'-NTA of uridines are more prone to be located in the exosomal fraction while 3'-NTA of adenines are rather found inside the cell. However, the mechanism involved in the differential localization of miRNAs is not addressed in this work. It has been also recently suggested that the cellular levels of miRNAs and their mRNA targets, at least in part, may regulate miRNA sorting into exosomes. Overexpression of cellular miRNAs or miRNA target sequences were able to modulate the presence of miRNAs in exosomes (Squadrito et al. 2014).

EV-miRNA modulation of target cells requires prior vesicle internalization by mechanisms still poorly understood. Different mechanisms have been described to be involved in EV-miRNA uptake such as clathrin-mediated endocytosis (Tian et al. 2014), phagocytosis (Feng et al. 2010) or macropinocytosis (Fitzner et al. 2011). Regardless of the pathway involved in EV-miRNA internalization, all processes should converge in the release of EVs lumen content into the acceptor cell cytoplasm in order to modulate gene expression.

Interestingly, several reports demonstrate that EVs derived from both immune and non-immune cells have immunoregulatory functions, driving either activation or suppression of the immune response (Robbins and Morelli 2014). This has led to intense research initially focused on EVs protein content rather than on their lipidic or genetic cargo. The immunostimulatory role of EVs has been widely established in the context of both direct and indirect MHC-antigen presentation but also EVs appear to have the capacity of directly stimulate effector immune cells such as NK cells, B cells or macrophages. On the other hand, immunosuppressive functions of EVs have been investigated in the context of tumour and viral immune evasion (Thery et al. 2009).

II MiRNAs and the Immune Response

MiRNAs are non-coding small RNA molecules of ~19-24 nucleotides capable of interfering with messenger RNAs (mRNAs) translation, promoting fine-tuning of gene expression, with paramount functions in the regulation of most biological processes. Their biogenesis consists of various cleavage steps mediated by exonucleases to yield a mature form of ~22 nucleotide double strand miRNA that will assemble into the RNA-induced silencing complex (RISC) to exert its mRNA repressing function. The RISC complex loaded with one single strand miRNA (referred to as either -3p or -5p) will scan the 3'-untranslated region of mRNA in seek of partially complementary sites inhibiting mRNA translation or promoting its degradation. This way each individual miRNA is capable of suppressing multiple mRNA targets while one mRNA can be targeted by many miRNAs at the same time. The nomenclature of miRNAs has evolved since their discovery; referring to the -3p or -5p strands of the hairpin loop that give rise to mature miRNA is the currently recommended in the field. However, most of the studies discussed in this review do not use this terminology, therefore miRNAs will be referred to as they appear in the original studies.

Studies using mice deficient for genes involved in the miRNA biogenesis pathway, such as the exonucleases Dicer and/or Drosha, provided the first piece of evidence for the central role of miRNAs in the regulation of the immune response. These studies revealed that miRNAs control both development and homeostasis of the immune system (Cobb et al. 2005, Muljo et al. 2005, Chong et al. 2008). Subsequent studies have identified the function of individual miRNAs such as miR-181a, miR-155, miR-223 or miR-146a during lymphocyte differentiation and activation (Li et al. 2007, Rodriguez et al. 2007, Johnnidis et al. 2008, Lu et al. 2010). Since then, the number of studies describing not only new functions for known miRNAs but also identifying novel miRNAs implicated in the immune response has experimented an explosive growth. MiRNAs can influence the development and function of immune cells either through the inhibition of transcription factors or by targeting key signalling molecules downstream of cellular receptors. In order to mount a proper immune response, avoiding autoimmunity or exacerbated inflammation, immune cells undergo throughout their life cycle numerous processes of differentiation, maturation and activation that require a tight regulation. The rapid control and fine-tuning of protein synthesis, renders miRNAs attractive candidates to exert these functions. Indeed, miRNA expression is dynamic and undergoes dramatic changes during T cell development and differentiation and their expression inversely correlates with the activation status of the cells being highest in resting naive and memory T cells and decreasing in activated effector cells (Bronevetsky et al. 2013). As an example, miR-125b, which is highly expressed in human naive CD4⁺ T cells and down-regulated during differentiation towards effector T cell subsets, directly regulates the expression of genes such as IFN- γ , IL-2 receptor- β , IL-10 receptor- α and the transcriptional repressor Blimp-1 (Rossi et al. 2011). In innate immune cells, miR-146a is rapidly upregulated in human monocytes stimulated with LPS and acts as a negative regulator of TLR signalling (Brudecki et al. 2013).

Currently, it has become evident that miRNAs regulate many aspects of the immune response. Patients affected by autoimmune diseases exhibit differential patterns of miRNA expression compared to healthy individuals, suggesting a causative role for the pathogenesis

of autoimmune diseases such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus, juvenile arthritis or autoimmune colitis among others (Ceribelli et al. 2012). Noteworthy specific miRNAs like miR-146a and miR-155 appear to be systematically dysregulated in these conditions (O'Connell et al. 2010, Divekar et al. 2011, Kurowska-Stolarska et al. 2011, Zhou et al. 2014).

In addition to their activity in the cytoplasm, miRNAs can be secreted to the extracellular environment by controlled, active and specific processes. Extracellular miRNAs can circulate in human plasma either assembled into non-vesicular ribonucleoprotein complexes, which confer them stability, associated with high and low-density lipoproteins (Vickers and Remaley 2012) or packaged into EVs. However, it has been suggested that the fraction of miRNAs associated to lipoproteins is not efficiently transferred to bystander cells (Wagner et al. 2013). EVs protect miRNAs from degradation during systemic transport and permit their delivery to specific recipient cells. It is not yet clear whether the majority of circulating miRNAs are present in EVs (Zhang et al. 2010, Gallo et al. 2012) or associated to ribonucleoprotein complexes (Arroyo et al. 2011, Turchinovich et al. 2011). Differences due to technical approaches for EV and RNA purification could account for these controversial results, but it is becoming undoubtedly clear that specific miRNAs are strongly associated with exosomes.

Remarkably, most of the studies published on the functional transfer of EV-miRNAs only address miRNA effects on its corresponding target and do not explore the biological relevance in the context of the immune response. Recently, a controversial work has been published challenging the role of EVs as vehicles for functional modulatory miRNAs under physiological conditions. This work proposes that the stoichiometry of miRNAs contained in EVs is not sufficient for target gene modulation (Chevillet et al. 2014). Although many studies use supraphysiological levels of EVs and therefore their biological significance may be taken cautiously, several alternative approaches using knock-out mice and adoptive transfer experiments support the physiological function of EV-miRNAs, as reviewed here.

The delivery of miRNAs shuttled in EVs to recipient cells can be achieved through cell contact-independent or direct cell contact-dependent mechanisms (during the immunological synapse) (Mittelbrunn et al. 2011). Besides the modulation of gene expression mediated by miRNA targeting, an alternative mechanism involving miRNA direct binding and activation of Toll-like receptors (TLRs) has been proposed to contribute to the regulation of the immune response (Fabbri et al. 2012).

III Immune Modulation Mediated by Immune Cells Derived EV-MiRNAs

T lymphocytes

T lymphocytes, like most immune cells, release EVs constitutively, however, their secretion is increased following activation, including TCR signalling (Blanchard et al. 2002, Alonso et al. 2005, Mittelbrunn et al. 2011, van der Vlist et al. 2012). Indeed, it has been recently reported that, upon stimulation, both B and T cells release exosomal miR-150, correlating with a downregulation of its intracellular levels (de Candia et al. 2013). Remarkably, this was observed not only *in vitro* but also *in vivo* after vaccination of humans with influenza

virus, where increased serum levels of EV-miR-150 correlated with a higher immune response. Experiments with mice depleted of mature CD4⁺ T cells demonstrated that T lymphocytes were the main source of EV-miR-150 (de Candia et al. 2013).

T cells play a key role in orchestrating adaptive immune responses, and specific miRNAs have been shown to be critical for the fitness and function of different T cell subsets (Baumjohann and Ansel 2013, Jeker and Bluestone 2013). Very interestingly, differences are not only found between exosomes and parental cells miRNomes (Mittelbrunn et al. 2011). It has been recently demonstrated that exosomes released by different effector T cell subsets, in particular Th1, Th2 and Treg cells have different miRNA signatures (Okoye et al. 2014). These differences are likely to contribute to EV-miRNA effector immune functions. Since cytokine microenvironment is capable of shaping the miRNome of immune cells, it is conceivable that the miRNA content of T cell-derived EVs may depend on the immune context to promote either tolerance or immunity.

Regarding the functional role of EV-miRNAs in T lymphocytes, Mittelbrunn et al. showed that T cells transfer exosomal miRNA to the antigen-presenting cell (APC) during the immune synapse (Mittelbrunn et al. 2011) Table and Figure. Moreover, EV-delivered overexpressed exogenous miRNAs were functional in the recipient cell, downregulating target genes, suggesting that the T cell may be modulating the APC function through the specific delivery of miRNAs at the early stages of immune recognition.

There has been much speculation about the function of EV-miRNAs *in vivo* and the ability of these molecules to mediate effects at a distance. In this regard, two interesting works have been very recently published, suggesting regulatory functions for T cell-derived EV-miRNAs *in vivo*. First, in a model of contact sensitivity it was reported that CD8⁺ suppressive T cells from tolerized mice release EVs capable of suppressing effector T cells (Bryniarski et al. 2013). Either intraperitoneal or oral administration of isolated suppressive EVs, induced a marked and maintained suppression of the inflammatory response *in vivo*. Moreover, these suppressive functions were abolished by anti-miR150, suggesting its involvement in the induction of tolerance in this model (Table and Figure). This was confirmed with miR-150 deficient mice since EVs isolated from these mice failed to induce immunosuppression. Another thought-provoking finding of this work was that the suppressive effects observed *in vivo* were antigen-specific as confirmed by dual reciprocal antigen criss-cross experiments. Noteworthy, this antigen-specificity was conferred by light-chain antibody coating of EVs, presumably coming from B cells that were antigen-specifically activated during tolerogenesis. This study offers new insights for therapeutic approaches using cell-targeted delivery of specific EV-delivered miRNA cargo.

A second study demonstrated that regulatory T (Treg) cells release exosomes containing both mRNAs and miRNAs different from those of other effector T cells (Okoye et al. 2014). Using Dicer knock-out mice devoided of mature miRNAs, the authors identified specific miRNAs transferred from Treg to conventional T cells. Interestingly, both Treg cells deficient for Dicer or for Rab27 (necessary for proper exosomal release) failed to suppress Th1 cells, strongly suggesting that both miRNAs and exosomes are required for Treg suppressive functions. MiRNA let-7d, alone or in combination with other transferred

miRNAs was able to reproduce the regulatory phenotype. The effects observed *in vitro* were also validated in an *in vivo* model of colonic inflammation where EV-mediated transfer of let-7d contributed to the suppression of pathogenic Th1 cells and prevented inflammation (Table and Figure). This study provides solid evidence of a specific EV-miRNA that contributes to Treg suppressive functions *in vivo*.

B Lymphocytes

B lymphocytes have been largely studied for their capacity to produce antibodies, but also exhibit important functions in antigen presentation and have paramount regulatory functions in the immune response (Mauri and Bosma 2012).

The release of exosomes by B cells involved in antigen-specific MHC-class II restricted T cell responses *in vivo* (Raposo et al. 1996) and its regulation have been largely studied (Muntasell et al. 2007). Besides antigen presentation, B cell-exosomes have been implicated in viral transmission and cell signalling (McLellan 2009). On the other hand, numerous studies on the role of miRNAs in the development and function of these cells have been published so far (de Yebenes et al. 2013). However, it was not until 2010 when Pegtel et al. provided solid evidence of the functional transfer of EV-miRNAs derived from B cells in the context of infection, as discussed below (Pegtel et al. 2010).

More recently, it has been demonstrated that primary B lymphocytes can be genetically programmed for the biogenesis and delivery of anti-sense sequences against miRNA (anti-miRNAs). Primary B lymphocytes transfected with plasmid DNA encoding anti-miR-150, efficiently released anti-miR-bearing EVs that were internalized by CD8+ T cells during cross-priming not only *in vitro* but also *in vivo*. Interestingly, although anti-miR-150 synthesized by B cells was secreted both as free and EV-packed fractions, only those associated with EVs were apparently taken up by CD8+ T cells, supporting the importance of EVs as carriers of functional miRNAs (Almanza et al. 2013). B cell-derived exosomes have also proven to be useful as carriers to deliver anti-miR-155 to macrophage cell lines, downmodulating endogenous miRNA in the recipient cell. Intravenous administration of miR-155 loaded exosomes into miR-155 deficient mice also resulted in the delivery of this miRNA to the liver (Momen-Heravi et al. 2014) Table and Figure.

In a more physiological context, it was reported that miR-155 is overexpressed in B cells from both individuals with monoclonal B-cell lymphocytosis and patients with chronic lymphocytic leukemia (CLL). Moreover, miR-155 was identified in circulating EVs from these patients (Ferrajoli et al. 2013). It has been suggested that miR-155 could be involved in the transition from monoclonal B cell lymphocytosis to chronic lymphocytic leukemia. Recent studies indicate a correlation between the levels of miR-155-3p expression and the responsiveness of malignant cells to chemotherapy *in vitro* (Fonte et al. 2013). Whether EV-miR-155 is involved in some immunological changes that occur in this condition is yet unknown. Interestingly, higher levels of EV-miR-155 in patients before treatment correlated with worse prognosis and poor response to therapy (Ferrajoli et al. 2013).

Taken together, the data available support that B lymphocytes may prove useful as a platform for synthesis and delivery of EV-miRNA modulators for immune therapy. However

evidence for a specific role of B cell-derived EV-miRNAs for modulation of immune responses is scarce.

Monocytes/ Macrophages

The majority of peripheral blood microvesicles are derived from platelets, and mononuclear phagocytes are the second most abundant producing population (Hunter et al. 2008). Monocytes and macrophages have crucial roles in tissue homeostasis and during pathological processes. It is important to highlight that the understanding of these populations has been recently challenged by the identification of new subsets and their development and function have been re-visited (Jenkins and Hume 2014).

Increasing evidence supports the role of macrophages as a source of EV-miRNAs that can be transferred to a set of cells, including cancer cells, endothelial cells or monocytes. Similar to B cells, genetic manipulation of macrophages highlights the capacity of these cells to deliver functional EV-miRNAs. Using chemically-modified miRNA it was demonstrated that, after transfection, the macrophage cell line THP-1 releases EVs containing exogenous miRNAs. Although the presence of modified miRNAs in different tissues after intravenous injection of THP-1 transfected cells was observed, it is not clear whether miRNAs navigated via THP-1 cells or associated with EVs (Akao et al. 2011).

In addition to the release of exogenously provided miRNAs, THP-1 are able to package endogenous miRNAs into EVs and release them in response to several stimuli. THP-1 cells in culture release EVs that contain miR-150 that can be transferred to the human microvascular endothelial cell line HMEC-I, promoting cell migration. *In vivo* studies confirmed that intravenous injection of THP-1-EVs significantly increases the level of miR-150 in mouse blood vessels. In this work, it was also observed that EVs isolated from the plasma of patients with atherosclerosis with higher levels of miR-150 promote HMEC-1 cell migration with a higher efficiency than EVs from healthy donors (Zhang et al. 2010). In this regard, the *in vivo* role of EVs collected from plasma of atherosclerosis patients in angiogenesis has been recently reported (Li et al. 2013).

Regarding the physiological relevance of cell-cell communication mediated by macrophage-derived EV-miRNAs, the clearest example corresponds to effects on cancer cells (Table and Figure). MiRNAs transfected into M2 macrophages can be shuttled into breast cancer cells packaged into exosomes in the absence of cell-cell contact and promote tumour invasiveness probably by targeting the Mef2c- β -catenin pathway (Yang et al. 2011). The role of macrophage-derived miRNAs has also been described in hepatocellular carcinoma, although in this case the transfer of miRNAs required cellular contact and gap junctions. It thus seems plausible that the mechanisms of miRNA uptake depend on the recipient cell. Also, it could be argued that the source of miRNAs (either exogenous or endogenous) may influence its fate.

More recently, it has been reported that normal macrophages produce microvesicles that are transported to diverse target cells, including monocytes, endothelial cells, epithelial cells, and fibroblasts (Ismail et al. 2013). MiRNA expression in macrophage-derived EVs was

studied in GM-CSF-stimulated monocytes, identifying miR-223 as the most abundant population, followed by miR-191. Using a luciferase reporter assay authors showed that miR-223 transferred from macrophage-EVs is functional in the recipient cell (Ismail et al. 2013).

Dendritic Cells

Dendritic cells (DCs) are potent APCs with important immunomodulatory functions in adaptive responses. While immature DCs downmodulate T cell activity promoting immune tolerance, mature DCs trigger immune responses by activating effector T lymphocytes, inducing their proliferation and differentiation (Morelli and Thomson 2007). DC-to-DC communication has been shown to be required for amplification of T cell-mediated immunogenic and/or tolerogenic responses. Several mechanisms have been implicated in this intercellular communication, including soluble mediators, trogocytosis (Harshyne et al. 2001, Herrera et al. 2004), nanotubules (Watkins and Salter 2005), apoptotic cell-derived vesicles (Albert et al. 1998) and exosome-delivered antigenic peptides during cognate immune recognition (Thery et al. 2002a, Morelli et al. 2004).

A mechanism of exosome-shuttled endogenous miRNAs that mediates communication between mouse DCs has been reported. DCs-derived exosomes exhibited a different pattern of miRNA expression according to the cells maturation stage. Exosomal miRNAs (specifically miR-148a and miR-451) were delivered to the cytosol of recipient cells where they were capable of repressing mRNA targets, suggesting an horizontal propagation mechanism mediated by EV-miRNAs (Montecalvo et al. 2012) Table and Figure.

Although the effects of DC-exosomes have been widely studied, little is known about their miRNA immunoregulatory functions and to date there is no evidence for EV-miRNA dependent cross-talk with non-DC cells.

Mast Cells

Mast cells (MCs) are tissue-resident cells that play a key role in protecting the host against a range of parasitic or bacterial infections. Also, they are able to cause detrimental inflammatory responses to allergens and exacerbated autoimmunity (Skokos et al. 2002). As other immune cells, MCs are both source and target of EVs.

It is known that exosomes derived from MCs are able to induce functional changes in T cells, B cells and DCs, however these effects have been attributed to the exosomes protein content (Skokos et al. 2001, Skokos et al. 2003). On the other hand, the role of miRNAs in MC biology is a growing field of research (Montagner et al. 2013). Regarding cell-cell communication through EV-miRNAs in MCs, it is important to mention that transfer of exosomal miRNAs and the first evidence of their functionality in recipient cells was first described in this cell type (Valadi et al. 2007). Additional studies have determined the miRNA profile in exosomes from MCs and confirmed EV-miRNA transfer to other MCs and human hematopoietic progenitor CD34+ cells (Skokos et al. 2001, Eldh et al. 2010, Ekstrom et al. 2012). Although the functional role of EV-miRNAs produced by MCs has been

explored in the context of oxidative stress, the putative role in immune response modulation remains unknown.

Other Immune Cells

Like other immune cells, NK cells are sensitive to EVs released by other cell types; however, very little is known about NK-derived exosomes, despite the importance of these cells in innate and adaptive immunity. Recently, it has been described that primary resting and activated NK cells, release exosomes expressing typical protein markers of NK cells and containing proteins with cytotoxic activity against several tumour cell lines and activated immune cells (Lugini et al. 2012). However, the miRNA content of EVs released by these cells is currently unknown.

Neutrophils have also been reported to release EVs with immunoregulatory functions. Ectosomes released from neutrophils have been shown to be capable of interfering with the maturation of monocyte-derived DCs (Eken et al. 2008) and a recent report demonstrates that neutrophil-EVs can mediate antibacterial effects (Timar et al. 2013). However, the characterization of neutrophil-EVs content has not been addressed and their role in immune responses is still poorly understood.

The scientific and clinical interest for mesenchymal stromal cells (MSCs) has experienced a tremendous growth for their potential use as therapeutic agents in different acute and chronic diseases. There is increasing evidence that the anti-inflammatory function of MSCs does not only rely on their ability to replace damaged tissues but also on paracrine effects mediated by their extracellular-secreted products, including EVs (Figuerola et al. 2014). The inhibition of effector T cell proliferation, blockade of DCs maturation and the stimulation of Treg cells proliferation are some of the immunomodulatory effects of MSCs, mediated at least in part by the secretion of IL-10, TGF- β and IDO among other soluble factors (Ren et al. 2009, Singer and Caplan 2011). Moreover, MSC-derived EVs have been reported to contribute to tissue repair in an animal model of stroke (Xin et al. 2012). In this work authors reported that neurite growth depends on EV-miR-133b transfer from MSCs to neurons and astrocytes. Although a growing number of reports suggest the ability to MSCs to secrete functional EV-miRNAs indicating their potential therapeutic use, their role in immunoregulation remains elusive. In a study comparing immunomodulatory effects of MSCs with EVs derived from the same MSCs, EVs showed a lower effect on T cell proliferation and antibody production compared with their cellular counterpart (Conforti et al. 2014). Very recently the exosome-enriched fraction from MSC supernatants were administrated in a patient with graft-versus-host disease (GvHD). Clinical improvements were observed shortly after the start of MSC-exosomes therapy and no side effects were registered (Kordelas et al. 2014).

IV Immune Modulation Mediated by Non-Immune Cells Derived EV-MiRNAs

Tumour Cells

Tumour-derived EVs have led to intense research in the last few years, since evidence has accumulated suggesting their involvement in promoting immune evasion, eliciting both

primary tumour growth and metastasis (Taylor and Gercel-Taylor 2011, Filipazzi et al. 2012).

Tumour-derived EV-miRNAs have been detected in body fluids from patients suffering different types of cancer. The minimum invasive methods required for their isolation and their correlation with prognosis and response to therapy have pointed towards tumour-released EV-miRNAs as promising diagnosis and risk biomarkers in cancer. Moreover, some of the miRNAs contained in exosomes derived from cancer cell lines, e.g. miR-146a, miR-29a or miR-21, have known functions for lymphocyte development and are present at sufficient levels to provoke fine-tuning of target genes (Cereghetti and Lee 2014).

Exosomes derived from glioblastoma cells contained both miRNAs and mRNAs coding for different angiogenic factors that could be translated into protein in the acceptor cell (Skog et al. 2008). This finding represented one of the first pieces of evidence of tumour-derived EVs as multicomponent carriers, not only delivering immunomodulatory proteins, but also communicating genetic information to modulate the function of bystander cells and tuning the tumour microenvironment. EV-miRNAs produced by different types of cancer cells, such as hepatocellular carcinoma (Kogure et al. 2011), renal cancer stem cells (Grange et al. 2011) or an acute monocytic leukemia cell line (Zhang et al. 2010) have been shown to downregulate mRNA targets in the recipient cell.

Tumour cells use different mechanisms to evade immune surveillance in order to grow and metastasize; EV-miRNAs are emerging as important players in these processes. Exosomes derived from nasopharyngeal carcinoma have been shown to facilitate tumour immune evasion by impairing T cell functions, inhibiting T cell proliferation and preventing the differentiation to effector subsets (Th1/Th17) while inducing Treg production (Ye et al. 2014). This exosome-mediated modulation of the T cell compartment induces changes in cytokine production and correlates with increased levels of five common miRNAs: miR-24-3p, miR-891a, miR-106a-5p, miR-20a-5p and miR-1908. These miRNA clusters are predicted to downregulate the MAPK1 signalling pathway, that may be involved in the alteration observed in T cell development and differentiation. However the direct role of miRNAs in this process has not been addressed.

Until very recently, it was believed that EV-delivered miRNAs exerted their function exclusively by downmodulating mRNA targets on the acceptor cell, however, a recent work from Fabbri et al. demonstrated that tumour secreted EV-miRNAs could mediate modulatory functions by direct binding to toll-like receptors (TLRs) (Fabbri et al. 2012). Hence miR-29a and miR-21, released in exosomes from lung cancer cell lines, were able to bind to human TLR8 (or its equivalent in mouse TLR7), but not to human TLR7 on macrophages, inducing a prometastatic inflammatory response (Table and Figure).

Although there is growing evidence of tumour-derived EVs modifying the tumour microenvironment to promote its growth and create a pre-metastatic niche, the mechanisms by which tumour secreted EV-miRNAs contribute to dampen immune surveillance are far from being fully understood.

Viral Infections

Viruses are able to hijack the cellular machinery of infected cells to counteract host immune responses. In particular, viral miRNAs are able to target specific cellular mRNAs promoting immune evasion and are involved in the regulation of the transition from latent to lytic replication of the virus (Cullen 2009). Pegtel and coworkers showed that B cells infected with Epstein-Barr virus (EBV) release exosomes containing viral miRNAs (Pegtel et al. 2010). These exosomes bearing miRNA BHRF1-3 could be internalized by monocyte-derived DCs, leading to specific repression of confirmed EBV-targets, such as the immunostimulatory CXCL11/ITAC gene (Table and Figure). Moreover, viral BamH1 rightward transcripts (BART) clusters of miRNAs were found both in peripheral B and non-B cells, suggesting the exosomal transfer of functional virus-derived miRNAs from infected cells to bystander cells *in vivo*. Furthermore, viral miR-BART5 has been shown to be transferred from infected B cells via exosomes and inhibit the NLRP3 inflammasome in non-infected cells (Table and Figure), presumably upon exosome caveolae-dependent endocytosis (Nanbo et al. 2013). This inhibition involves a reduction in the expression of the NLRP3 protein, by targeting its coding mRNA, and in IL-1 β production (Haneklaus et al. 2012). The virus takes advantage of the cellular endogenous homeostatic miR-223-mediated mechanism to limit the NLRP3 inflammatory capacity during myeloid cell development.

Hepatitis C virus (HCV) infected cells also release RNA-containing exosomes that can be transferred to plasmacytoid DCs, triggering IFN- α production (Table). This mechanism of exosomal export of viral RNA has been proposed to represent both a host viral-clearance strategy to induce innate responses in cells that are non-permissive for viral replication but also a virus mechanism to evade immune surveillance (Dreux et al. 2012).

Trophoblasts and Pregnancy

Placental trophoblasts have critical regulatory functions at the materno-foetal interface to ensure transport functions and hormone production, while protecting the foetus from infections. Recently, miRNAs have been shown to play an important role in the regulation of homeostasis during pregnancy and placental specific clusters have been detected in maternal blood that rapidly disappear after delivery (Ng et al. 2003, Gilad et al. 2008). The miRNA cluster located in chromosome 19 (C19MC), normally silenced in healthy cells except for embryonic stem cells and placenta, represents a high proportion of this miRNA repertoire (Bentwich et al. 2005, Donker et al. 2012).

Placenta releases EVs, both exosomes and microvesicles and, interestingly, there is a time-dependent occurrence of both species, exosomes being mainly released during the first trimester of pregnancy and microvesicles being detectable from the second trimester and increasing as pregnancy progresses. Balance between these two types of vesicles has been shown to be critical for pre-eclampsia (Ouyang et al. 2014, Record 2014).

Interestingly, human placental trophoblasts have been shown to release exosomes that contain placental specific miRNAs, enriched in C19MC species, that endow non-placental recipient cells with resistance to viral infection by promoting autophagy (Delorme-Axford et al. 2013, Mouillet et al. 2014) Table and Figure. The antiviral effect can be abolished by

depleting exosomes and is reproduced when transfecting cells with C19MC highly expressed miRNAs. However, the cellular mRNA targets have not been identified yet.

Since C19MC miRNAs have also been detected in thyroid adenomas and adenomatous goiters, it has been recently proposed that C19MC, more specifically the delivery of these miRNAs via EVs to bystander cells, may have immunomodulatory functions in materno-foetal communication, conferring viral protection to the foetus, and similarly may provide a growth advantage to thyroid nodules by protecting them from autoimmune attacks (Bullerdiek and Flor 2012).

Interestingly, immunocompetent exosomes have been shown to be present in breast milk (both human and bovine) and it has been hypothesized that milk exosome-delivered miRNA, like miR-155, may promote long-term lineage commitment of Foxp3+ T regulatory cells and decrease of IL-4/Th2-mediated atopic sensitization (Melnik et al. 2014). In support of this hypothesis, several reports show that unboiled farm milk intake protects from the development of atopy in infants and has been shown to be associated with an increase of Treg cells. Furthermore, breast milk contains exosomes, enriched in miRNA species, and these EVs have an immunoregulatory role, being capable of promoting Foxp3+ Treg differentiation *in vitro*, although the involvement of miRNAs in this induction has not been addressed (Admyre et al. 2007). Altogether these observations suggest that EV-miRNAs play a pivotal role in orchestrating all phases of reproduction.

Other Non-Immune Cell Types

As mentioned above, platelet-derived EVs are the most abundant species among circulating vesicles. Their abundance and the relevance of platelets in inflammatory responses, mainly studied in atherosclerotic disease, besides their well-known role in homeostasis and wound repair after vascular injury, support the interest of studying platelet-EVs. Platelets express both miRNAs and the associated processing machinery, including Ago2, TRBP2 and DICER molecules (Landry et al. 2009). Although the platelet miRNA content is low compared to other cells, miRNA profiles of platelets and plasma exhibit a high degree of correlation, suggesting a platelet origin for an important fraction of the circulating miRNAs. Platelet-derived microparticles (PMP) have been identified in joint fluid from patients with rheumatoid arthritis, and are capable of inducing the production of proinflammatory cytokines in fibroblast-like synoviocytes through a mechanism that involves IL-1 (Boilard et al. 2010). These findings suggest that platelets may participate and amplify inflammatory responses in the pathogenesis of arthritis through the release of proinflammatory microparticles. Interestingly, several miRNAs with important known functions in the immune response are highly represented in platelet-derived microparticles, including miR-155, miR-150, miR-27a, miR-19 or miR-21 (Willeit et al. 2013).

Recent studies have shown that endothelial cells deliver EVs that contain miRNAs with atheroprotective functions, e.g. miR-143/145 transferred via exosomes to smooth muscle cells (Hergenreider et al. 2012). Moreover, horizontal transfer of miR-126 from endothelial cells to recipient bystander cells via apoptotic bodies has been shown to promote vascular tissue repair by inducing CXCL12 production (Zernecke et al. 2009) Table and Figure. Similarly, it was demonstrated that activated platelets release microparticles bearing mRNA

regulatory Ago2-miR-223 complexes to endothelial cells (Laffont et al. 2013) where they modulated gene expression. Although in a non-immune context, these data support the role of EV-miRNAs released from platelets and endothelial cells as mediators of cell-cell communication.

Future Perspectives: Diagnosis, Prognosis, Therapy

Although the importance of EV-miRNAs as a novel platform for cell communication has only recently come into the focus of the scientific community, increasing evidence is revealing their role in the regulation of numerous biological processes. It is now undoubted that genetic information released by the cells to the extracellular environment as “messages in bottles” shuttled inside EVs has biological significance and impacts on recipient cells. The technical difficulties of studying the EV-miRNA transfer *in vivo* constitute a major challenge to fully understand the EV-miRNA function *in vivo*. In particular, discerning the effects mediated by transferred exogenous miRNA from those due to endogenous miRNA upregulation following exosome-cell interactions or cytokine-mediated effects is hard to approach. Indeed, many studies do not rule out the putative effects of other immuno-modulatory molecules, that could be modified during the experimental procedures. Two different approaches have been used to discriminate the origin of miRNAs; artificial miRNA overexpression (Mittelbrunn et al. 2011) and the genetic modification of recipient cells to ablate the machinery necessary for miRNA biogenesis and therefore the majority of endogenous miRNAs (Okoye et al. 2014). Currently, a limited number of studies have clearly shown a functional impact of EV-miRNA transfer in the immune system (summarized in Table and Figure). Conceivably, this field will experience an enormous growth in the coming years.

The mechanisms by which miRNAs are selectively sorted into EVs are only beginning to be understood. Recent studies suggest an involvement of specific nucleotide motifs (Villarroya-Beltri et al. 2013), specific nucleotide modifications (Koppers-Lalic et al. 2014) or protein interactions for the inclusion of miRNAs into EVs. Identifying the natural interplayers of EVs loading is crucial for the use of these vesicles as carriers of genetic information and/or active proteins that could act as therapeutic agents in a wide range of pathologies. Furthermore, antibody-coating of EVs has been shown to direct the vesicles towards a specific cellular compartment *in vivo* (Bryniarski et al. 2013). Engineering both to modify EVs content and to direct them towards a specific subset of recipient cells, may prove an extremely powerful tool to fight against many pathologies from infections to tumours or autoimmune diseases.

The use of EVs as carriers of small RNA for therapy has been reported. EVs used as a tool to delivering antisense RNA against miR-150 in an animal model of tumour showed an antiangiogenic effect through the regulation of VEGF (miR-150 target) expression (Liu et al. 2013). Very recent data also indicate the potential value of EVs as a strategy to treat human diseases. Based on the evidence that immuno-regulatory factors of MSCs reside in the supernatant fraction, enriched in EVs, MSCs-exosomes were used to treat a patient with GvHD. The beneficial effects registered after the administration of MSC-exosomes

(Kordelas et al. 2014) open the possibility of using EVs as a novel therapeutic weapon to treat not only GvHD but other inflammatory diseases.

EVs and their genetic content are also emerging as promising biomarkers for diagnosis and prognosis for a range of diseases. EVs isolation from plasma and other body fluids like urine is much more accessible than other conventional procedures, making their analysis suitable for routine examinations. EVs content reflects the source and status of their producing cells, representing powerful elements to identify markers like proteins and miRNAs that will reflect pathologies, for example an incipient tumour (Skog et al. 2008, Willeit et al. 2013).

In sum, the biology of miRNAs carried by EVs and their function in the context of immune cell communication in homeostatic and pathological conditions is far from being understood. However, a lot of efforts are being made to acquire a better knowledge of EV-miRNA transfer processes that may pave the way for novel therapeutic approaches based on targeted delivery of specific EV-miRNAs for directed immune modulation.

Acknowledgments

Work in the authors' laboratories are supported by grants SAF2011-25834, ERC-2011-ADG_20110310, Cardiovascular Network RD12-0042-0056 (Instituto de Salud Carlos III), PIE-13-00041 and INDISNET S2011-BMD-2332. L.F.M. is supported by the Juan de la Cierva grant (Spanish Ministry of Economy and Competitiveness) and E.R.G. by the Spanish Ministry of Health. We would like to thank Dr. Hugh T. Reyburn for critically reading the manuscript.

References

- Admyre C, Johansson SM, Qazi KR, Filen JJ, Lahesmaa R, Norman M, Neve EP, Scheynius A, Gabrielsson S. Exosomes with immune modulatory features are present in human breast milk. *J Immunol.* 2007; 179(3):1969–1978. [PubMed: 17641064]
- Akao Y, Iio A, Itoh T, Noguchi S, Itoh Y, Ohtsuki Y, Naoe T. Microvesicle-mediated RNA molecule delivery system using monocytes/macrophages. *Mol Ther.* 2011; 19(2):395–399. [PubMed: 21102562]
- Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature.* 1998; 392(6671):86–89. [PubMed: 9510252]
- Almanza G, Anufreichik V, Rodvold JJ, Chiu KT, DeLaney A, Akers JC, Chen CC, Zanetti M. Synthesis and delivery of short, noncoding RNA by B lymphocytes. *Proc Natl Acad Sci U S A.* 2013; 110(50):20182–20187. [PubMed: 24277816]
- Alonso R, Rodriguez MC, Pindado J, Merino E, Merida I, Izquierdo M. Diacylglycerol kinase alpha regulates the secretion of lethal exosomes bearing Fas ligand during activation-induced cell death of T lymphocytes. *J Biol Chem.* 2005; 280(31):28439–28450. [PubMed: 15870081]
- Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A.* 2011; 108(12):5003–5008. [PubMed: 21383194]
- Baumjohann D, Ansel KM. MicroRNA-mediated regulation of T helper cell differentiation and plasticity. *Nat Rev Immunol.* 2013; 13(9):666–678. [PubMed: 23907446]
- Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, et al. Identification of hundreds of conserved and nonconserved human microRNAs. *Nat Genet.* 2005; 37(7):766–770. [PubMed: 15965474]
- Blanchard N, Lankar D, Faure F, Regnault A, Dumont C, Raposo G, Hivroz C. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J Immunol.* 2002; 168(7):3235–3241. [PubMed: 11907077]

- Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, Massarotti EM, Remold-O'Donnell E, Farndale RW, Ware J, Lee DM. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science*. 2010; 327(5965):580–583. [PubMed: 20110505]
- Bronevetsky Y, Villarino AV, Easley CJ, Barbeau R, Barczak AJ, Heinz GA, Kremmer E, Heissmeyer V, McManus MT, Erle DJ, Rao A, et al. T cell activation induces proteasomal degradation of Argonaute and rapid remodeling of the microRNA repertoire. *J Exp Med*. 2013; 210(2):417–432. [PubMed: 23382546]
- Brudecki L, Ferguson DA, McCall CE, El Gazzar M. MicroRNA-146a and RBM4 form a negative feed-forward loop that disrupts cytokine mRNA translation following TLR4 responses in human THP-1 monocytes. *Immunol Cell Biol*. 2013; 91(8):532–540. [PubMed: 23897118]
- Bryniarski K, Ptak W, Jayakumar A, Pullmann K, Caplan MJ, Chairoungdua A, Lu J, Adams BD, Sikora E, Nazimek K, Marquez S, et al. Antigen-specific, antibody-coated, exosome-like nanovesicles deliver suppressor T-cell microRNA-150 to effector T cells to inhibit contact sensitivity. *J Allergy Clin Immunol*. 2013; 132(1):170–181. [PubMed: 23727037]
- Bullerdiek J, Flor I. Exosome-delivered microRNAs of "chromosome 19 microRNA cluster" as immunomodulators in pregnancy and tumorigenesis. *Mol Cytogenet*. 2012; 5(1):27. [PubMed: 22559272]
- Cereghetti DM, Lee PP. Tumor-Derived Exosomes Contain microRNAs with Immunological Function: Implications for a Novel Immunosuppression Mechanism. *Microna*. 2014; 2(3):194–204. [PubMed: 25069443]
- Ceribelli A, Satoh M, Chan EK. MicroRNAs and autoimmunity. *Curr Opin Immunol*. 2012; 24(6): 686–691. [PubMed: 22902047]
- Cobb BS, Nesterova TB, Thompson E, Hertweck A, O'Connor E, Godwin J, Wilson CB, Brockdorff N, Fisher AG, Smale ST, Merkenschlager M. T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer. *J Exp Med*. 2005; 201(9):1367–1373. [PubMed: 15867090]
- Conforti A, Scarsella M, Starc N, Giorda E, Biagini S, Proia A, Carsetti R, Locatelli F, Bernardo ME. Microvesicles Derived from Mesenchymal Stromal Cells Are Not as Effective as Their Cellular Counterpart in the Ability to Modulate Immune Responses In Vitro. *Stem Cells Dev*. 2014; doi: 10.1089/scd.2014.0091
- Cullen BR. Viral RNAs: lessons from the enemy. *Cell*. 2009; 136(4):592–597. [PubMed: 19239880]
- Chevillet JR, Kang Q, Ruf IK, Briggs HA, Vojtech LN, Hughes SM, Cheng HH, Arroyo JD, Meredith EK, Gallichotte EN, Pogossova-Agadjanyan EL, et al. Quantitative and stoichiometric analysis of the microRNA content of exosomes. *Proc Natl Acad Sci U S A*. 2014; 111(41):14888–14893. [PubMed: 25267620]
- Chong MM, Rasmussen JP, Rudensky AY, Littman DR. The RNaseIII enzyme Drosha is critical in T cells for preventing lethal inflammatory disease. *J Exp Med*. 2008; 205(9):2005–2017. [PubMed: 18725527]
- de Candia P, Torri A, Gorletta T, Fedeli M, Bulgheroni E, Cheroni C, Marabita F, Crosti M, Moro M, Pariani E, Romano L, et al. Intracellular modulation, extracellular disposal and serum increase of MiR-150 mark lymphocyte activation. *PLoS One*. 2013; 8(9):e75348. [PubMed: 24205408]
- de Yebenes VG, Bartolome-Izquierdo N, Ramiro AR. Regulation of B-cell development and function by microRNAs. *Immunol Rev*. 2013; 253(1):25–39. [PubMed: 23550636]
- Delorme-Axford E, Donker RB, Mouillet JF, Chu T, Bayer A, Ouyang Y, Wang T, Stolz DB, Sarkar SN, Morelli AE, Sadovsky Y, et al. Human placental trophoblasts confer viral resistance to recipient cells. *Proc Natl Acad Sci U S A*. 2013; 110(29):12048–12053. [PubMed: 23818581]
- Divekar AA, Dubey S, Gangalum PR, Singh RR. Dicer insufficiency and microRNA-155 overexpression in lupus regulatory T cells: an apparent paradox in the setting of an inflammatory milieu. *J Immunol*. 2011; 186(2):924–930. [PubMed: 21149603]
- Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, Sadovsky Y. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. *Mol Hum Reprod*. 2012; 18(8):417–424. [PubMed: 22383544]

- Dreux M, Garaigorta U, Boyd B, Decembre E, Chung J, Whitten-Bauer C, Wieland S, Chisari FV. Short-range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity. *Cell Host Microbe*. 2012; 12(4):558–570. [PubMed: 23084922]
- Eken C, Gasser O, Zenhausern G, Oehri I, Hess C, Schifferli JA. Polymorphonuclear neutrophil-derived ectosomes interfere with the maturation of monocyte-derived dendritic cells. *J Immunol*. 2008; 180(2):817–824. [PubMed: 18178820]
- Ekstrom K, Valadi H, Sjostrand M, Malmhall C, Bossios A, Eldh M, Lotvall J. Characterization of mRNA and microRNA in human mast cell-derived exosomes and their transfer to other mast cells and blood CD34 progenitor cells. *J Extracell Vesicles*. 2012; doi: 10.3402/jev.v1i0.18389
- Eldh M, Ekstrom K, Valadi H, Sjostrand M, Olsson B, Jernas M, Lotvall J. Exosomes communicate protective messages during oxidative stress; possible role of exosomal shuttle RNA. *PLoS One*. 2010; 5(12):e15353. [PubMed: 21179422]
- Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, Zanesi N, et al. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proc Natl Acad Sci U S A*. 2012; 109(31):E2110–2116. [PubMed: 22753494]
- Feng D, Zhao WL, Ye YY, Bai XC, Liu RQ, Chang LF, Zhou Q, Sui SF. Cellular internalization of exosomes occurs through phagocytosis. *Traffic*. 2010; 11(5):675–687. [PubMed: 20136776]
- Ferrajoli A, Shanafelt TD, Ivan C, Shimizu M, Rabe KG, Nouraei N, Ikuo M, Ghosh AK, Lerner S, Rassenti LZ, Xiao L, et al. Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia. *Blood*. 2013; 122(11):1891–1899. [PubMed: 23821659]
- Figueroa FE, Cuenca Moreno J, La Cava A. Novel approaches to lupus drug discovery using stem cell therapy. Role of mesenchymal-stem-cell-secreted factors. *Expert Opin Drug Discov*. 2014; 9(5): 555–566. [PubMed: 24655067]
- Filipazzi P, Burdek M, Villa A, Rivoltini L, Huber V. Recent advances on the role of tumor exosomes in immunosuppression and disease progression. *Semin Cancer Biol*. 2012; 22(4):342–349. [PubMed: 22369922]
- Fitzner D, Schnaars M, van Rossum D, Krishnamoorthy G, Dibaj P, Bakhti M, Regen T, Hanisch UK, Simons M. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J Cell Sci*. 2011; 124(Pt 3):447–458. [PubMed: 21242314]
- Fonte E, Apollonio B, Scarfo L, Ranghetti P, Fazi C, Ghia P, Caligaris-Cappio F, Muzio M. In vitro sensitivity of CLL cells to fludarabine may be modulated by the stimulation of Toll-like receptors. *Clin Cancer Res*. 2013; 19(2):367–379. [PubMed: 23224401]
- Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One*. 2012; 7(3):e30679. [PubMed: 22427800]
- Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, et al. Serum microRNAs are promising novel biomarkers. *PLoS One*. 2008; 3(9):e3148. [PubMed: 18773077]
- Gould SJ, Raposo G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. *J Extracell Vesicles*. 2013; doi: 10.3402/jev.v2i0.20389
- Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, Camussi G. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer Res*. 2011; 71(15):5346–5356. [PubMed: 21670082]
- Haneklaus M, Gerlic M, Kurowska-Stolarska M, Rainey AA, Pich D, McInnes IB, Hammerschmidt W, O'Neill LA, Masters SL. Cutting edge: miR-223 and EBV miR-BART15 regulate the NLRP3 inflammasome and IL-1 β production. *J Immunol*. 2012; 189(8):3795–3799. [PubMed: 22984081]
- Harshyne LA, Watkins SC, Gambotto A, Barratt-Boyes SM. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. *J Immunol*. 2001; 166(6):3717–3723. [PubMed: 11238612]
- Hergenreider E, Heydt S, Treguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, et al. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol*. 2012; 14(3):249–256. [PubMed: 22327366]

- Herrera OB, Golshayan D, Tibbott R, Salcido Ochoa F, James MJ, Marelli-Berg FM, Lechler RI. A novel pathway of alloantigen presentation by dendritic cells. *J Immunol.* 2004; 173(8):4828–4837. [PubMed: 15470023]
- Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, Nana-Sinkam SP, et al. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One.* 2008; 3(11):e3694. [PubMed: 19002258]
- Ismail N, Wang Y, Dakhllallah D, Moldovan L, Agarwal K, Batte K, Shah P, Wisler J, Eubank TD, Tridandapani S, Paulaitis ME, et al. Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. *Blood.* 2013; 121(6):984–995. [PubMed: 23144169]
- Jeker LT, Bluestone JA. MicroRNA regulation of T-cell differentiation and function. *Immunol Rev.* 2013; 253(1):65–81. [PubMed: 23550639]
- Jenkins SJ, Hume DA. Homeostasis in the mononuclear phagocyte system. *Trends Immunol.* 2014; 35(8):358–367. [PubMed: 25047416]
- Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, Brummelkamp TR, Fleming MD, Camargo FD. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature.* 2008; 451(7182):1125–1129. [PubMed: 18278031]
- Kogure T, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. *Hepatology.* 2011; 54(4):1237–1248. [PubMed: 21721029]
- Koppers-Lalic D, Hackenberg M, Bijnsdorp IV, van Eijndhoven MA, Sadek P, Sie D, Zini N, Middeldorp JM, Ylstra B, de Menezes RX, Wurdinger T, et al. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. *Cell Rep.* 2014; 8(6):1649–1658. [PubMed: 25242326]
- Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doeppner TR, Eppele M, Horn PA, Beelen DW, Giebel B. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. *Leukemia.* 2014; 28(4):970–973. [PubMed: 24445866]
- Kurowska-Stolarska M, Alivernini S, Ballantine LE, Asquith DL, Millar NL, Gilchrist DS, Reilly J, Ierna M, Fraser AR, Stolarski B, McSharry C, et al. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. *Proc Natl Acad Sci U S A.* 2011; 108(27):11193–11198. [PubMed: 21690378]
- Laffont B, Corduan A, Ple H, Duchez AC, Cloutier N, Boilard E, Provost P. Activated platelets can deliver mRNA regulatory Ago2*microRNA complexes to endothelial cells via microparticles. *Blood.* 2013; 122(2):253–261. [PubMed: 23652806]
- Landry P, Plante I, Ouellet DL, Perron MP, Rousseau G, Provost P. Existence of a microRNA pathway in anucleate platelets. *Nat Struct Mol Biol.* 2009; 16(9):961–966. [PubMed: 19668211]
- Lehmann SM, Kruger C, Park B, Derkow K, Rosenberger K, Baumgart J, Trimbuch T, Eom G, Hinz M, Kaul D, Habel P, et al. An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration. *Nat Neurosci.* 2012; 15(6):827–835. [PubMed: 22610069]
- Li J, Zhang Y, Liu Y, Dai X, Li W, Cai X, Yin Y, Wang Q, Xue Y, Wang C, Li D, et al. Microvesicle-mediated transfer of microRNA-150 from monocytes to endothelial cells promotes angiogenesis. *J Biol Chem.* 2013; 288(32):23586–23596. [PubMed: 23766514]
- Li QJ, Chau J, Ebert PJ, Sylvester G, Min H, Liu G, Braich R, Manoharan M, Soutschek J, Skare P, Klein LO, et al. miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell.* 2007; 129(1):147–161. [PubMed: 17382377]
- Liu Y, Zhao L, Li D, Yin Y, Zhang CY, Li J, Zhang Y. Microvesicle-delivery miR-150 promotes tumorigenesis by up-regulating VEGF, and the neutralization of miR-150 attenuate tumor development. *Protein Cell.* 2013; 4(12):932–941. [PubMed: 24203759]
- Lu LF, Boldin MP, Chaudhry A, Lin LL, Taganov KD, Hanada T, Yoshimura A, Baltimore D, Rudensky AY. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. *Cell.* 2010; 142(6):914–929. [PubMed: 20850013]
- Lugini L, Cecchetti S, Huber V, Luciani F, Macchia G, Spadaro F, Paris L, Abalsamo L, Colone M, Molinari A, Podo F, et al. Immune surveillance properties of human NK cell-derived exosomes. *J Immunol.* 2012; 189(6):2833–2842. [PubMed: 22904309]

- Mauri C, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol.* 2012; 30:221–241. [PubMed: 22224776]
- Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res.* 2010; 107(9):1047–1057. [PubMed: 21030722]
- McLellan AD. Exosome release by primary B cells. *Crit Rev Immunol.* 2009; 29(3):203–217. [PubMed: 19538135]
- Melnik BC, John SM, Schmitz G. Milk: an exosomal microRNA transmitter promoting thymic regulatory T cell maturation preventing the development of atopy? *J Transl Med.* 2014; 12:43. [PubMed: 24521175]
- Mittelbrunn M, Gutierrez-Vazquez C, Villarroya-Beltri C, Gonzalez S, Sanchez-Cabo F, Gonzalez MA, Bernad A, Sanchez-Madrid F. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat Commun.* 2011; 2:282. [PubMed: 21505438]
- Momen-Heravi F, Bala S, Bukong T, Szabo G. Exosome-mediated delivery of functionally active miRNA-155 inhibitor to macrophages. *Nanomedicine.* 2014; doi: 10.1016/j.nano.2014.03.014
- Montagner S, Orlandi EM, Merante S, Monticelli S. The role of miRNAs in mast cells and other innate immune cells. *Immunol Rev.* 2013; 253(1):12–24. [PubMed: 23550635]
- Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan ML, Karlsson JM, Baty CJ, Gibson GA, Erdos G, Wang Z, Milosevic J, et al. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood.* 2012; 119(3):756–766. [PubMed: 22031862]
- Morelli AE, Larregina AT, Shufesky WJ, Sullivan ML, Stolz DB, Papworth GD, Zahorchak AF, Logar AJ, Wang Z, Watkins SC, Falo LD Jr, et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood.* 2004; 104(10):3257–3266. [PubMed: 15284116]
- Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat Rev Immunol.* 2007; 7(8):610–621. [PubMed: 17627284]
- Moreno-Gonzalo O, Villarroya-Beltri C, Sanchez-Madrid F. Post-translational modifications of exosomal proteins. *Front Immunol.* 2014; 5:383. [PubMed: 25157254]
- Mouillet JF, Ouyang Y, Bayer A, Coyne CB, Sadovsky Y. The role of trophoblastic microRNAs in placental viral infection. *Int J Dev Biol.* 2014; 58(2-3-4):281–289. [PubMed: 25023694]
- Muljo SA, Ansel KM, Kanelloupolou C, Livingston DM, Rao A, Rajewsky K. Aberrant T cell differentiation in the absence of Dicer. *J Exp Med.* 2005; 202(2):261–269. [PubMed: 16009718]
- Muntasell A, Berger AC, Roche PA. T cell-induced secretion of MHC class II-peptide complexes on B cell exosomes. *EMBO J.* 2007; 26(19):4263–4272. [PubMed: 17805347]
- Nanbo A, Kawanishi E, Yoshida R, Yoshiyama H. Exosomes derived from Epstein-Barr virus-infected cells are internalized via caveola-dependent endocytosis and promote phenotypic modulation in target cells. *J Virol.* 2013; 87(18):10334–10347. [PubMed: 23864627]
- Ng EK, Tsui NB, Lau TK, Leung TN, Chiu RW, Panesar NS, Lit LC, Chan KW, Lo YM. mRNA of placental origin is readily detectable in maternal plasma. *Proc Natl Acad Sci U S A.* 2003; 100(8):4748–4753. [PubMed: 12644709]
- Nolte-t Hoen EN, Buermans HP, Waasdorp M, Stoorvogel W, Wauben MH, t Hoen PA. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. *Nucleic Acids Res.* 2012; 40(18):9272–9285. [PubMed: 22821563]
- O'Connell RM, Kahn D, Gibson WS, Round JL, Scholz RL, Chaudhuri AA, Kahn ME, Rao DS, Baltimore D. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. *Immunity.* 2010; 33(4):607–619. [PubMed: 20888269]
- Okoye IS, Coomes SM, Pelly VS, Czieso S, Papayannopoulos V, Tolmachova T, Seabra MC, Wilson MS. MicroRNA-Containing T-Regulatory-Cell-Derived Exosomes Suppress Pathogenic T Helper 1 Cells. *Immunity.* 2014; 41(1):89–103. [PubMed: 25035954]
- Ouyang Y, Mouillet JF, Coyne CB, Sadovsky Y. Review: placenta-specific microRNAs in exosomes - good things come in nano-packages. *Placenta.* 2014; 35(Suppl):S69–73. [PubMed: 24280233]
- Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, de Gruijl TD, Wurdinger T, Middeldorp JM. Functional delivery of viral miRNAs via exosomes. *Proc Natl Acad Sci U S A.* 2010; 107(14):6328–6333. [PubMed: 20304794]

- Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med*. 1996; 183(3):1161–1172. [PubMed: 8642258]
- Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol*. 2013; 200(4):373–383. [PubMed: 23420871]
- Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak MZ. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia*. 2006; 20(5):847–856. [PubMed: 16453000]
- Record M. Intercellular communication by exosomes in placenta: a possible role in cell fusion? *Placenta*. 2014; 35(5):297–302. [PubMed: 24661568]
- Ren G, Su J, Zhang L, Zhao X, Ling W, L'Huillier A, Zhang J, Lu Y, Roberts AI, Ji W, Zhang H, et al. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. *Stem Cells*. 2009; 27(8):1954–1962. [PubMed: 19544427]
- Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol*. 2014; 14(3):195–208. [PubMed: 24566916]
- Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, et al. Requirement of bic/microRNA-155 for normal immune function. *Science*. 2007; 316(5824):608–611. [PubMed: 17463290]
- Rossi RL, Rossetti G, Wenandy L, Curti S, Ripamonti A, Bonnal RJ, Birolo RS, Moro M, Crosti MC, Gruarin P, Maglie S, et al. Distinct microRNA signatures in human lymphocyte subsets and enforcement of the naive state in CD4+ T cells by the microRNA miR-125b. *Nat Immunol*. 2011; 12(8):796–803. [PubMed: 21706005]
- Simons M, Raposo G. Exosomes--vesicular carriers for intercellular communication. *Curr Opin Cell Biol*. 2009; 21(4):575–581. [PubMed: 19442504]
- Singer NG, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. *Annu Rev Pathol*. 2011; 6:457–478. [PubMed: 21073342]
- Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008; 10(12):1470–1476. [PubMed: 19011622]
- Skokos D, Botros HG, Demeure C, Morin J, Peronet R, Birkenmeier G, Boudaly S, Mecheri S. Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J Immunol*. 2003; 170(6):3037–3045. [PubMed: 12626558]
- Skokos D, Goubran-Botros H, Roa M, Mecheri S. Immunoregulatory properties of mast cell-derived exosomes. *Mol Immunol*. 2002; 38(16–18):1359–1362. [PubMed: 12217408]
- Skokos D, Le Panse S, Villa I, Rousselle JC, Peronet R, David B, Namane A, Mecheri S. Mast cell-dependent B and T lymphocyte activation is mediated by the secretion of immunologically active exosomes. *J Immunol*. 2001; 166(2):868–876. [PubMed: 11145662]
- Squadrito ML, Baer C, Burdet F, Maderna C, Gilfillan GD, Lyle R, Ibberson M, De Palma M. Endogenous RNAs Modulate MicroRNA Sorting to Exosomes and Transfer to Acceptor Cells. *Cell Rep*. 2014; doi: 10.1016/j.celrep.2014.07.035
- Taylor DD, Gercel-Taylor C. Exosomes/microvesicles: mediators of cancer-associated immunosuppressive microenvironments. *Semin Immunopathol*. 2011; 33(5):441–454. [PubMed: 21688197]
- Thery C, Duban L, Segura E, Veron P, Lantz O, Amigorena S. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. *Nat Immunol*. 2002a; 3(12):1156–1162. [PubMed: 12426563]
- Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol*. 2009; 9(8):581–593. [PubMed: 19498381]
- Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. 2002b; 2(8):569–579. [PubMed: 12154376]
- Tian T, Zhu YL, Zhou YY, Liang GF, Wang YY, Hu FH, Xiao ZD. Exosome Uptake through Clathrin-mediated Endocytosis and Macropinocytosis and Mediating miR-21 Delivery. *J Biol Chem*. 2014; 289(32):22258–22267. [PubMed: 24951588]

- Timar CI, Lorincz AM, Csepanyi-Komi R, Valyi-Nagy A, Nagy G, Buzas EI, Ivanyi Z, Kittel A, Powell DW, McLeish KR, Ligeti E. Antibacterial effect of microvesicles released from human neutrophilic granulocytes. *Blood*. 2013; 121(3):510–518. [PubMed: 23144171]
- Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. *Nucleic Acids Res*. 2011; 39(16):7223–7233. [PubMed: 21609964]
- Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007; 9(6):654–659. [PubMed: 17486113]
- van der Vlist EJ, Arkesteijn GJ, van de Lest CH, Stoorvogel W, Nolte-'t Hoen EN, Wauben MH. CD4(+) T cell activation promotes the differential release of distinct populations of nanosized vesicles. *J Extracell Vesicles*. 2012; doi: 10.3402/jev.v1i0.18364
- Vickers KC, Remaley AT. Lipid-based carriers of microRNAs and intercellular communication. *Curr Opin Lipidol*. 2012; 23(2):91–97. [PubMed: 22418571]
- Villarroya-Beltri C, Baixauli F, Gutierrez-Vazquez C, Sanchez-Madrid F, Mittelbrunn M. Sorting it out: Regulation of exosome loading. *Semin Cancer Biol*. 2014; doi: 10.1016/j.semcancer.2014.04.009
- Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F, Perez-Hernandez D, Vazquez J, Martin-Cofreces N, Martinez-Herrera DJ, Pascual-Montano A, Mittelbrunn M, Sanchez-Madrid F. Sumoylated hnRNP A2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat Commun*. 2013; 4:2980. [PubMed: 24356509]
- Wagner J, Riwanto M, Besler C, Knau A, Fichtlscherer S, Roxe T, Zeiher AM, Landmesser U, Dimmeler S. Characterization of levels and cellular transfer of circulating lipoprotein-bound microRNAs. *Arterioscler Thromb Vasc Biol*. 2013; 33(6):1392–1400. [PubMed: 23559634]
- Watkins SC, Salter RD. Functional connectivity between immune cells mediated by tunneling nanotubules. *Immunity*. 2005; 23(3):309–318. [PubMed: 16169503]
- Willeit P, Zampetaki A, Dudek K, Kaudewitz D, King A, Kirkby NS, Crosby-Nwaobi R, Prokopi M, Drozdov I, Langley SR, Sivaprasad S, et al. Circulating microRNAs as novel biomarkers for platelet activation. *Circ Res*. 2013; 112(4):595–600. [PubMed: 23283721]
- Witwer KW, Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, Nolte-'t Hoen EN, Piper MG, Sivaraman S, Skog J, Thery C, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J Extracell Vesicles*. 2013; doi: 10.3402/jev.v2i0.20360
- Xin H, Li Y, Buller B, Katakowski M, Zhang Y, Wang X, Shang X, Zhang ZG, Chopp M. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells*. 2012; 30(7):1556–1564. [PubMed: 22605481]
- Yang M, Chen J, Su F, Yu B, Su F, Lin L, Liu Y, Huang JD, Song E. Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol Cancer*. 2011; 10:117. [PubMed: 21939504]
- Ye SB, Li ZL, Luo DH, Huang BJ, Chen YS, Zhang XS, Cui J, Zeng YX, Li J. Tumor-derived exosomes promote tumor progression and T-cell dysfunction through the regulation of enriched exosomal microRNAs in human nasopharyngeal carcinoma. *Oncotarget*. 2014; 5(14):5439–5452. [PubMed: 24978137]
- Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M, Koppel T, Jahantigh MN, Lutgens E, Wang S, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal*. 2009; 2(100):ra81. [PubMed: 19996457]
- Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y, Li J, Bian Z, Liang X, Cai X, Yin Y, et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Res*. 2012; 22(1):107–126. [PubMed: 21931358]
- Zhang Y, Liu D, Chen X, Li J, Li L, Bian Z, Sun F, Lu J, Yin Y, Cai X, Sun Q, et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell*. 2010; 39(1):133–144. [PubMed: 20603081]
- Zhou Q, Haupt S, Kreuzer JT, Hammitzsch A, Proft F, Neumann C, Leipe J, Witt M, Schulze-Koops H, Skapenko A. Decreased expression of miR-146a and miR-155 contributes to an abnormal Treg phenotype in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2014; doi: 10.1136/annrheumdis-2013-204377

Functional transfer of EV-miRNAs in the immune system.

miRNAs secreted into EVs (exosomes, microvesicles and apoptotic bodies) from both immune and non-immune cells can be transferred to recipient cells where they are able to modulate gene expression. This figure summarizes the functional outcome observed after EV-mediated delivery of miRNAs (endogenous and/or exogenous[†]) that mediate either tolerance or immunity.

EV-delivered miRNAs in the immune system

Table 1

miRNA	Donor cell	Recipient cell	EV	Target gene/Pathway	Function	Reference
IMMUNE CELL-DERIVED EV-MIRNAS	miR-335 ϕ miR-92a	T cell line (J77c120)	B cell line (Raji)	Exosomes	SOX4	Immune synapse dependent functional transfer of miRNAs (Mittelbrunn, Gutierrez-Vazquez et al. 2011)
	miR-150	Suppressive CD8+ T cell	Effector T cell	Exosomes	N.A.	Suppression of effector T cells (Bryniarski, Ptak et al. 2013)
	let-7d	Treg cell	Th1 cell	Exosomes	Cox-2 IFN- γ	Suppression of Th1 pathogenic cell proliferation and IFN- γ production (Okoye, Coomes et al. 2014)
	anti-miRNA-150 ϕ	B cell	CD8+ T cell	Exosomes	miR-150	Inhibition of inflammation (Almanza, Anufreichik et al. 2013)
	miR-155 ϕ	B cell	Macrophage	Exosomes	SOCS1 mRNA TNF- α	Inhibition of LPS-induced pro-inflammatory cytokine production (Momen-Heravi, Bala et al. 2014)
	miR-223	Macrophage	Breast Cancer cell	Exosomes	Mef2c- β -catenin pathway	Tumour invasiveness (Yang, Chen et al. 2011)
	miR-223	Macrophage	Monocyte Endothelial Cell Epithelial Cell Fibroblast	Exosomes	Hematologic system development	Macrophage differentiation and survival (Ismail, Wang et al. 2013)
	miR-148a miR-451	DC	DC	Exosomes	N.A.	DC-DC communication (dependent on cell maturation state) (Montecalvo, Larregina et al. 2012)
	miR-21 miR-29a	Lung cancer cell	Macrophage	Exosomes	Direct activation human TLR8 mice TLR7	Prometastatic inflammatory response (Fabbri, Paone et al. 2012)
	miR-BHRF1-3	EBV-infected B cells	Monocyte-derived DC	Exosomes	CXCL11/ITAC	Repression of immunostimulatory factors (Pegtel, Cosmopoulos et al. 2010)
NON-IMMUNE CELL-DERIVED EV-MIRNAS	miR-223	EBV-infected B cell	Non-infected cell	Exosomes	NLRP3 inflammasome IL1- β	Limitation of NLRP3 inflammasome and pro-inflammatory cytokine production (Haneklaus, Gerlic et al. 2012)
	HCV-RNA	HCV-infected cell	Plasmaeytoid DC	Exosomes	IFN- α	Activation of innate immunity (Dreux, Garaigorta et al. 2012)
	CM19MC miR-517-3p miR-516b-5p miR-512-3p	Trophoblast	Non-placental cell	Exosomes	Autophagy	Viral resistance Induction of autophagy (Delorme-Axford, Donker et al. 2013)
	miR-126	Endothelial cell	Endothelial cell Aortic cell Smooth muscle cell	Apoptotic bodies	CXCL12	Tissue repair (Zernecke, Bidzhekov et al. 2009)

ϕ Exogenous miRNAs
N.A. Not addressed