Review

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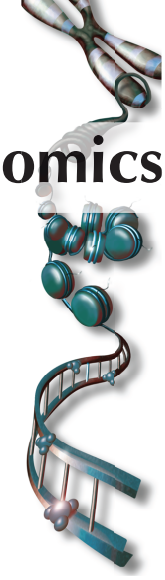
Epigenomics



Exosomal miRNAs: novel players in viral infection

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Exosomes are secreted nanovesicles that are able to transfer their cargo (such as miRNAs) between cells. To determine to what extent exosomes and exosomal miRNAs are involved in the pathogenesis, progression and diagnosis of viral infections. The scientific literature (PubMed and Google Scholar) was searched from 1970 to 2019. The complex biogenesis of exosomes and miRNAs was reviewed. Exosomes contain both viral and host miRNAs that can be used as diagnostic biomarkers for viral diseases. Viral proteins can alter miRNAs, and conversely miRNAs can alter the host response to viral infections in a positive or negative manner. It is expected that exosomal miRNAs will be increasingly used for diagnosis, monitoring and even treatment of viral infections.

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Exosomes are double-membrane vesicles of about 30–150 nm in diameter, with a composition of lipids and proteins and are enriched with lipid raft-associated molecules. In addition to lipids and proteins, exosomes also contain nucleic acid molecules, such as DNA, mRNA, viral genomic nucleic acids and miRNAs [1,2]. These exosomal RNAs can be taken up by neighboring or distant cells, and subsequently modulate behavior of the recipient cells [3]. The discovery of the biological function of exosomes, where by genetic material can be exchanged between cells has attracted increasing attention. miRNAs are a class of small noncoding RNAs that play multiple regulatory roles in a range of cellular processes, such as apoptosis, immune function, tumorigenesis, and they also can both suppress and contribute to virus replication [4–6]. Several virus families have been demonstrated to encode miRNAs in their genome, and these can carry out important activities including contributing to evasion of the host immune response, regulation of viral gene expression, promotion of survival of infected cells, and a possible role in the maintenance of latent and persistent infection [7,8]. MiRNAs are selectively packaged, released and transferred between cells encapsulated in exosomes. miRNAs are actively released by exosomes, which protect them from degradation by RNAses [9]. Viruses use several mechanisms to evade from and suppress the host immune system in order to ensure their replication and persistence. However, the immune system utilizes a counteracting mechanism for removal of pathogens [10,11]. Viruses have evolved to insert their viral components into exosomes that exert several downstream effects by a variety of mechanisms [11]. There have been numerous studies that have demonstrated functional transfer of exosomal miRNAs between cells both *in vitro* and *in vivo*. The functional transfer of exosomes into recipient

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cells leads to the facilitation of virus replication through inhibiting immune responses. It has been found that some viruses are able to alter the packaging of the miRNAs into the exosomes, suggesting that these alterations could be beneficial for viral infections [11–13]. Exosomal miRNAs have been found to be present in synovial fluid, breast milk, urine, blood and saliva. Their amount and compositions differ between healthy and virally infected cells. Therefore, exosomal miRNAs may play an important role as noninvasive markers for monitoring and diagnosis of viral diseases. In this review, we discuss the alteration of packaging and dysregulation of miRNAs in the exosomes derived from virus-infected cells, and outline the implications of these changes for viral infections.

Biogenesis of exosomes

Exosomes were discovered in the early 1980s by Stahl and Johnstone, during their studies on loss of transferrin during reticulocyte maturation [14]. These authors described exosomes to be complex extracellular vesicles (EVs) that were secreted by direct budding from the plasma membrane of cells, and could be isolated from human body fluids and also from cultured cells [15]. Several studies have demonstrated that these small vesicles initially formed via inward budding inside intracellular endosomes, go on to lead to the formation of multivesicular bodies (MVBs) [15]. MVBs are late endosomes, which contain intraluminal vesicles (ILVs) and have sizes ranging between 30 and 100 nm in diameter [14,16]. MVBs can either be degraded, or else can fuse with the plasma membrane, thus releasing ILVs into the extracellular environment. The ILVs released from the MVBs are called exosomes [17]. The biogenesis of exosomes is mediated via the endosomal sorting complex transport (ESCRT) machinery [18]. ESCRT machinery contains four different protein complexes: ESCRT-0, -I, -II, -III and also related AAA ATPase Vps4 complexes [19]. The most complete study of ESCRTs in exosomes biosynthesis, used an RNAi approach that targeted 23 ESCRT and ESCRT-related proteins in immortal human cells (HeLa cells) [20]. The exosomes that are released can be purified by attachment to anti-CD63 beads, and identified by means of anti-CD81 and anti-HLA-DR (MHC II) fluorescent antibodies using flow cytometry. Using shRNA transfection, seven ESCRT proteins were recognized in exosomes. Knockdown of the TSG101, ESCRT-0 protein Hrs and the ESCRT-I protein STAM1 inhibited the release of exosomes. Knockdown of ESCRT-III, and the related proteins VPS4B, CHMP4C, ALIX and VTA1 enhanced the release of exosomes. In studies of four proteins detected in exosomes by ultracentrifugation, it was found that reduction of Hrs, TSG101 and STAM1 diminished exosome release, while VPS4B reduction enhanced exosome release [20–22].

Knockdown of ALIX appeared to alter the protein composition of exosomes, rather than to affect their release. This could show that ALIX has an effect on cargo loading and/or the subtypes of MVBs, which have been designated for release. Researchers found that ALIX knockdown in dendritic cells (DCs) diminished exosome release in about half of the donors.

Moreover, in three different studies, the ESCRT-0 protein Hrs was found to be involved in release of exosomes [22,23]. DCs with knockdown of Hrs were found to release fewer exosomes, measured as the exosomal levels of ubiquitinated proteins, TSG101 and VPS4B. In another study [21], reduction of Hrs in HEK293 cells was found to diminish exosomal Evi and Wnt3A [22]. In agreement with this result, Camilleri and colleagues used nanoparticle tracking analysis to show that reduction of Hrs-diminished exosome release from head and neck squamous carcinoma cells [23].

Studies have indicated that membrane proteins containing heparan sulfate chains called syndecans, which are found within exosomes are regulated through their attachment to the syntenin–ALIX complex. Syntenin is a polyvalent soluble protein that can bind both ALIX and syndecans, thus creating a connection between syndecans and the ESCRT machinery [24]. The interplay between syntenin and ALIX also affects the composition of ILVs [24]. The same group also found that heparanases can trim the heparan sulfate side chains of syndecans, thereby assisting the formation of syndecan clusters, which could induce their attachment to syntenin [25]. Importantly, heparanase also induced the inclusion of CD63 [25]. The syndecan–syntenin–ALIX complex has been estimated to account for about 50% of the released vesicles in MCF-7 cells [26]. Studies have suggested that MVB biosynthesis could occur even when ESCRTs were absent. With the knockdown the subunits of each of the four ESCRT complexes, ILVs were still created in MVBs, showing the existence of ESCRT-unrelated pathways [27]. Tetraspanins are transmembrane proteins, which are abundant in exosomes, and may also be involved in exosome release [28]. The release of exosomes containing β-catenin from HEK293 cells, was shown to be related to the expression of the tetraspanins, CD9 and CD82.

It was shown that bone marrow DCs (BMDCs) isolated from CD9 knockout mice released less exosome-related flotillin-1. Another tetraspanin, involved in exosome biosynthesis is Tspan8 [29]. The expressing of Tspan8 in rat

adenocarcinoma cells affected the total number of released exosomes, with little effect on the mRNA and protein contents of the exosomes. The tetraspanin, CD63 has also been shown to be involved in exosome biosynthesis [30].

Researchers have shown that cells, which express Epstein–Barr virus (EBV)-encoded latent membrane protein 1 (LMP1) released more exosomes in comparison with cells without LMP1, and that this LMP-1 effect also involved CD63 [31]. The 'small integral membrane protein of the lysosome or late endosome' (SIMPLE) as well as the 'lipopolysaccharide-induced TNF factor' LITAF, are proteins that have been suggested to be important in exosome formation. After transfecting COS cells with SIMPLE (and mutated SIMPLE) an increased release of exosomes was detected [32]. As well as proteins, lipids have critical roles in vesicular transport [33]. Two kinds of lipid-related processes contribute to vesicular transport, namely, membrane fission and destruction, and membrane fusion [34]. Membrane deformation is governed by the type of membrane lipids, including the degree of saturation of the acyl chains, the size and length of the head group.

Many studies have indicated the participation of lipids in the exosome composition through lipid-regulating enzymes. Blocking nSMase2, the enzyme, which produces ceramide from sphingomyelin, decreases the release of exosomes containing proteolipid protein from Oli-neu cells [35]. A similar study showed that blocking nSMase2 decreased the secretion of exosomal EGFP-CD63 from EGFP-CD63-transfected PC-3 cells. The mechanisms for these effects are obscure, but it may be that ceramide microdomains coalescence in larger domains, which then enhance membrane budding [35]. Researchers have proposed that role of ceramide in exosome secretion is unlikely, due to their high-speed growth [36,37], but this does not apply to all the cell lines in which it was evaluated [38]. For instance, in PC-3 cells, exosomes are secreted via nSMase2, and blocking *de novo* ceramide production had no effect [38]. Phospholipase D2 (PLD2), which generates phosphatidic acid (PA) from phospholipids, is another lipid-regulating enzyme whose role has been assessed in exosome production. The action of this enzyme was related to the secretion of exosomes in RBL-2H3 cells [39]. PLD2 was proposed to function as a modulator of the small GTPase, ARF6 that has been recognized as a modulator of exosome biosynthesis and governs the composition of ILVs [40]. The direct involvement of PLD2-produced PA in exosome biosynthesis was not proposed. In another study, diacylglycerol kinase α (DGKα; an enzyme that adds a phosphate group to diacyl glycerol producing PA) was implicated in the secretion of exosomes from T lymphocytes [41]. However, DGKα was found to have a negative effect on the construction of mature MVBs [29]. Furthermore, DGKα was found to interact with protein kinase D1/2 to promote MVB maturation and exosome release [42]. As mentioned above, exosome biosynthesis can be divided into ESCRT-related and ESCRT-unrelated pathways [43], but these pathways might not be completely separate [44]. One pathway might act as an enhancer of the other pathway. In addition, the cell types and/or state of cellular homeostasis might be critical factors in exosome release.

MiRNA biogenesis

Most miRNA genes are found within the introns of protein encoding, as well as noncoding genes [45]. The majority of miRNAs are transcribed by RNA polymerase II, as long primary miRNAs. This process is regulated by the MEDIATOR complex. Furthermore, cyclin-dependent kinases such as CDKF1 and CDKDs phosphorylate the C-terminal domain of the largest subunit of RNA polymerase II [46]. Some transcription factors regulate the transcription of miRNA genes and miRNA-related pathways, for example, NOT2 interacts with RNA polymerase II, and regulates the transcription of some miRNA genes [46]. Primary miRNA (pri-miRNA) consists of a hairpin structure composed of a terminal loop and a stem region. Pri-miRNA is 5′-capped, spliced and polyadenylated, producing one (or more) functional miRNA molecule [47]. The RNase III enzymes, Dicer and Drosha specifically process these 'pri-miRNAs' to generate mature miRNA duplexes containing 19–24 nucleotides (Figure 1) [48]. In the nucleus, 'microprocessor', a multiprotein complex, first crops the pri-miRNAs into an approximately 70 nucleotide hairpin-structured precursor called pre-miRNA. Next Drosha and DGCR8/Pasha (which is a dsRNA-binding domain [dsRBD] protein) that are the two major components of the microprocessor, together with the cofactors DDX5, p72 (DDX17) and heterogeneous nuclear ribonucleoproteins (hnRNPs) [47] work together to cleave the double-stranded region of the pre-miRNA to produce a 2-nt 3′ overhang, which is recognized by XPO5 and promotes translocation of the pre-miRNA from the nucleus to the cytoplasm via a Ran-GTP-dependent mechanism [49,50]. In the cytoplasm, Dicer (another RNase III enzyme) working with the dsRBD proteins, TRBP/PACT, further cleaves the pre-miRNA into an approximately 22-nt long miRNA duplex.

One strand of the miRNA duplex, usually an A/G rich strand with a 5′-U initiation site called the guide strand binds to an Argonaute (AGO) protein within the RNA-induced silencing complex (RISC). The duplex passenger strand is U/C rich, and usually begins with a 5′-C, and is destined to be degraded. Based on expression

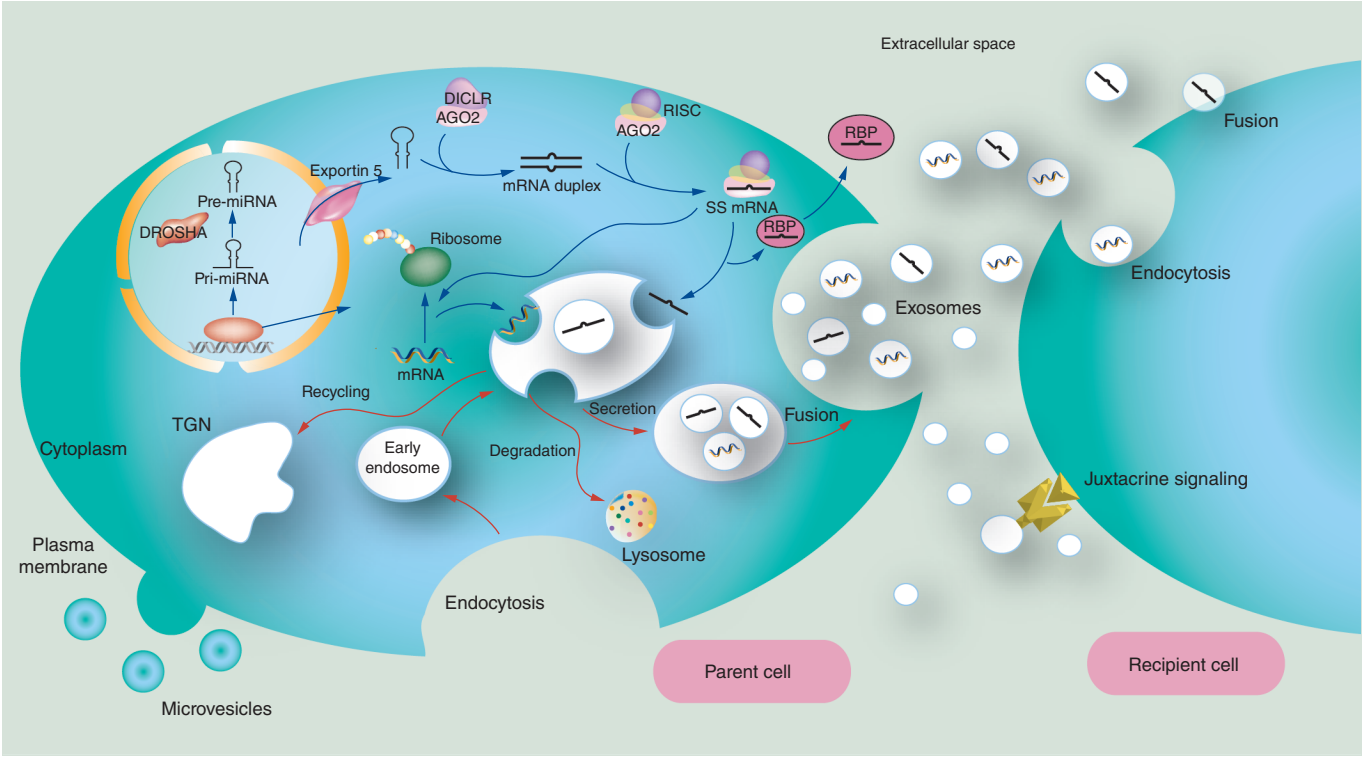

Figure 1. Biogenesis of exosomes in parent cells and uptake of exosomes by recipient cells. Showing how membrane pathways, vesicular pathways and mRNA/miRNA pathways are all involved.

profiling, both strands can be equally abundant in some tissues [48]. The miRNA guides the RISC to bind to the targeted mRNA sequence at the 3′UTR, thus destroying the mRNA, leading to dysregulation of the target gene expression [47,51].

Furthermore, some alternative Drosha-independent pathways of miRNAs processing have been found, such as tRNA- and snoRNA-derived miRNAs, and mirtrons. Although one strand of miRNA is loaded into Ago for mRNA binding leading to gene repression, by means of RNA-binding proteins (RBPs), the other strand is transported toward the plasma membrane for secretion in microvesicles, or else toward MVBs for secretion in exosomes. The assessment of the miRNA content of exosomes derived from cardiac fibroblasts, showed the abundance of several passenger strands of miRNA [52]. The absence of Ago2 from exosomes suggests that exosomal miRNAs are guided by RBPs, as well as protected against degradation [53].

RBPs play a role in the transportation of the miRNA strands either toward MVBs for loading into exosomes, or toward the plasma membrane for secretion. After the transportation of endosomes to the trans-Golgi network, they can be routed to lysosomes for degradation, or else interact with microtubules to bind to the plasma membrane, leading to release of ILVs as exosomes into the extracellular space. Exosomes are able to interact with recipient cells via membrane fusion, endocytosis or juxtacrine signaling. By the outward budding of the plasma membrane, the parent cells also release microvesicles into the extracellular space.

Lipid-induced loading of RNA into exosomes

Spontaneous inward budding of the raft-like areas of the membrane of MVBs creates ILVs, and the exosomes that are formed are enriched in phosphatidylcholine (PC), saturated fatty acids, glycosphingolipids, sphingomyelin and cholesterol (Figure 2). Loading of RNAs into exosomes, commences after binding of RNAs to the raft-like areas [54]. The binding of RNAs to the membranes is mediated through numerous factors, including sphingosine, lipid structures (especially lipid rafts) and hydrophobic modifications [55,56]. Some specific nucleotide sequences show increased affinity to the phospholipid bilayers [57–59]. Indeed, by combining such preselected RNA motifs with high membrane affinity along with an amino acid-binding RNA motif, a passive membrane transporter specific for the amino acid tryptophan was constructed and characterized [60]. The existence of interactions between the

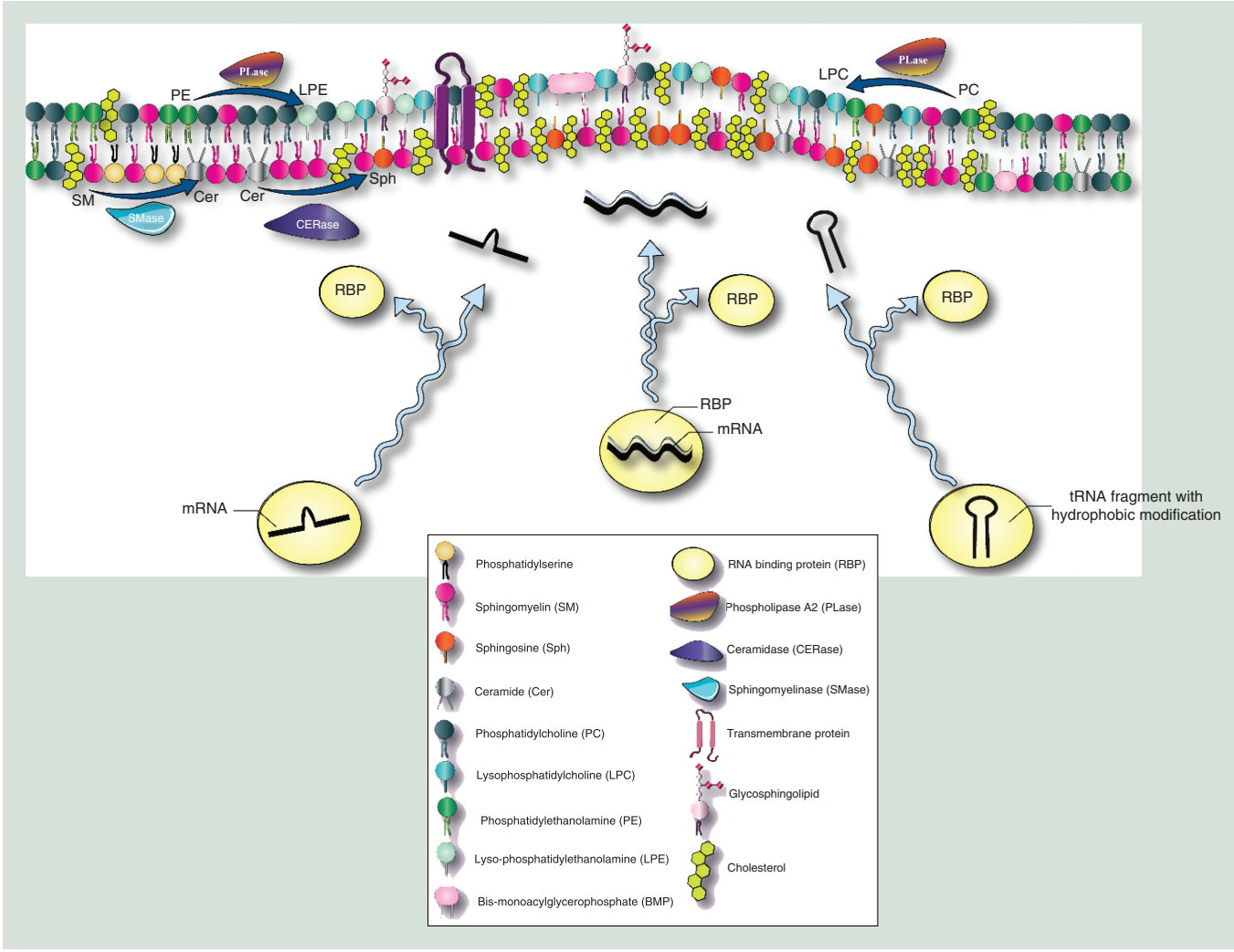

Figure 2. RNAs binding to raft-like areas of the cytoplasmic surface of the multivesicular body membrane. There is an interaction between the cytoplasmic leaflet of the MVB membrane and RNAs that are transported by RBPs. The highest affinity of RNAs to the raft-like areas occurs at the membrane. The affinity of the RNA molecule to the raft-like areas is governed by hydrophobic areas of RNA, and RNA sequence-dependent binding motifs. Both glycosphingolipid and lysophospholipid molecules present at the luminal surface, as well as ceramide molecules in the cytoplasmic membrane surface, mediate the spontaneous inward budding process. After fusion of the plasma membrane with MVBs, ILVs are generated leading to exosomes.
ILV: Intraluminal vesicle; MVB: Multivesicular body; RBP: RNA-binding protein.

cytoplasmic surface of the MVB membrane and some cellular RNAs has recently been suggested [54]. RNAs are selected based on their affinity to the raft-like areas in the MVB membrane in order to be incorporated into ILVs, but the budding process is independent of RNA binding [54].

Raft-like areas in the cytoplasmic surface of the MVB membrane

Because MVBs are an end-product of the endosomal pathway, their formation involves plasma membrane proteins, such as nSMase2, as well as lipids (including lipids specific for membrane rafts). nSMase2 is palmitoylated on several cysteine residues within the catalytic site. Sphingomyelin is broken down into ceramide and phosphocholine by nSMase2 at the inner plasma membrane surface [61]. Topologically, the external surface of the MVB membrane and the inner surface of the plasma membrane are equivalent. Therefore, ceramide molecules are produced at the MVB cytoplasmic membrane surface. nSMase2 directly and specifically interacts with PA and phosphatidylserine, and the nSMase2 enzymatic activity depends on these anionic phospholipids [62], which are enriched in exosomes and

raft-like areas [63,64]. Ceramide can be converted to S1P and sphingosine via sphingosine kinase and ceramidase [65]. The activation of S1P receptors on MVBs induces their maturation and transports tetraspanins into ILVs [65].

Ceramide is able to self-associate via hydrogen bonding, which provides the driving force for the coalescence of microscopic rafts into a larger macrodomain [66]. This macrodomain serves as a platform for the process of ILV budding [67]. The clustering of raft domains and the heterogeneity provided by ceramide, generate active platform regions within cell membranes [68]. In a study with liposomal membrane consisting of sphingomyelin and PC, sphingomyelinase causes domain clustering and the formation of membrane microdomains, which leads to subsequent small vesicles being shed from the membrane into the interior of large liposomes [69].

Lipid-mediated inward budding of the raft-like area of the MVB membrane

Purified exosomes are enriched in ceramide, and when nSMase is inhibited, exosomes release is decreased [70]. In fact, ceramide stimulates exosome secretion in mammalian cells, and a number of exosomal miRNAs are released via a secretory process, which is independent of the ESCRT system, and rather depends on ceramide activity [71]. To sort the cargo toward MVBs, an ESCRT independent pathway, working via raft-based microdomains, may laterally segregate the cargoes within the endosomal membrane [70].

It is likely that the enrichment of exosomes in lyso-phosphatidylethanolamine (lyso-PE) and lyso-PC is important for the budding process [63,72]. sPLA2-V plays a role in the recycling of endosomes and in the Golgi network [73]. sPLA2-V generates lyso-PE and lyso-PC inside the lumenal surface of MVB membranes. Dipalmitoyl PC is a major lipid raft component [74]. It can serve as a phospholipase A2 substrate and plays a stabilizing role in raft-like generation within the MVB membrane. Nonetheless, because of its cylindrical shape, it has not been implicated in the budding process.

The enrichment of exosomal membranes in glycosphingolipids has been documented. Due to the localization of glycosphingolipids at the external plasma membrane leaflet, they take up an inward orientation at the endosomal membrane, leading to their localization at the external surface of ILVs. Specific structures of oligosaccharides as well as the large hydrophilic head groups of glycosphingolipids, work together to stabilize lipid domains and subdomains into a positive curvature [75]. Due to their membrane orientation (toward the MVB lumen), glycosphingolipids are probably not implicated in the interaction of the MVB membrane and RNAs at the cytoplasmic side.

The cone-shaped structure of the ceramide in the raft-like areas of the cytoplasmic membrane surface, as well as the inverted cone-shaped structures of glycosphingolipids and lysophospholipids in the lumenal membrane surface induce a pronounced membrane curvature (Figure 2). This results in the process of budding from the MVB membrane, thus generating ILVs within MVBs. The topology of the budding area correlates with the topology of the plasma membrane. Due to sPLA2-V at the lumenal side, and nSMase 2 at the cytoplasmic side of the MVB membrane a larger curvature of the budding area can be attained. Because of the larger surface area of budding vesicles compared with the border region surface (Figure 2), the driving force for the budding process primarily comes from the raft-like areas. The opposite curvature of the border region requires cone-shaped lipids at the cytoplasmic leaflet of the border region. These cone-shaped lipids could move to the border region from the area of the MVB membrane outside the raft-like region, where lysophospholipids could be created from phospholipids by the action of cytosolic phospholipases [73].

In the MVB membrane, specific proteins are also able to locate in the raft-like areas [76]. These lipid rafts have been proposed to be the weak points on the membrane leaflet, which are more disposed to outward budding or bending [76].

RNA binding to the raft-like areas of the MVB membrane

In contrast to the proteins that are sorted into MVBs by ESCRT in an ubiquitin-dependent manner [77], the sorting of RNAs is ceramide-dependent and ESCRT-independent in mammalian cells [74]. Additionally, the sorting of miRNAs into exosomes is probably a passive process designed to dispose of excessive amounts of miRNAs that are not required for their cellular targets [75]. Since the raft-like areas involve ceramide, it has been suggested that specific RNA sorting is dependent on the differential affinity of certain RNA motifs to bind to the raft-like area of the cytoplasmic leaflet of the MVB membrane [78]. This affinity to the raft-like area has been previously demonstrated [79]. RNAs with a particular sequence and secondary structure may bind to the ordered-liquid domains in PC-cholesterol-sphingomyelin vesicles resembling the ordered-liquid domains in exosomal membranes [79,80]. Randomly arranged sequences of RNA possess 20-fold lower affinities to the raft regions. Moreover, to increase the affinity to phospholipid bilayers, particular nucleotide sequences are needed, which are not found in random

sequences of RNA. Likewise, mRNA fragments enriched in the 3′UTRs that involve sequence elements that determine the subcellular localization of mRNAs, are transported by exosomes released by human cells [55,81].

The presence of sphingosine increases the affinity of tRNA to membrane rafts [56]. In the MVB membrane, ceramide is converted to sphingosine via ceramidase. Consequently, in addition to its production in the raft-like areas, sphingosine may promote RNA affinity to the MVB membrane [82].

Specific exosome-sorting motifs in RNA have been recently proposed for both mRNA and miRNA (this has been called the EXOmotif) [83,84]. Remarkably, ten EXOmotifs (UGCC, GGCG, GGAC, two UCCG, two UGAC, three GCCG) have been found in the raft-binding sequence of RNA 67–2 [79]: GGGACGACGAUGACACGAUACUUUGUCGGCCGAACUCGCUGCUCCGAUCCGGCGAGAUCGCAGGGUGUUGCUAUUCGCGUGCCGUGUGCAUACGCCGAUCACAUGACCA [85].

Another 11 EXOmotifs (GGCC, CCCG, GGCG, GGAC, two UGCC, two UGAC, three GCCG) have been identified in another raft-binding sequence, RNA 10 [79]: GGGACGACGAUGACACGAUACUUUGUCGGCCGAACUCGCUGUUUAACUGCCCGGCGAGAUCGCAGGGUGUUGUGCUAUUCGCGUGCCGUGUGCAUACGCCGAUCACAUGACCA. It was found that, among the all the examined RNA sequences, RNA 10 and RNA 67–2 possessed the most powerful affinity to the raft-like region [79]. According to recent data, RBPs have an important role in miRNA delivery to the raft-like regions [54]. In fact, the exosomal release of miRNA is promoted by its sequence-dependent correlation with hnRNPA2B1 (heterogeneous nuclear ribonucleoprotein A2/B1) [84]. Interestingly, hnRNPA2B1 protein possesses a high affinity to ceramide-rich membrane regions [84] and can be detected in exosome independently of miRNAs. Ago2 is usually absent from exosomes, which suggests that, independently of the miRNA production process, preformed miRNAs are released in exosomes [53]. Due to the presence of miRNAs in a 13-fold excess over the amount of Agos in HeLa cells, there are excess miRNAs available to bind to the raft-like areas of MVBs [86].

According to recent data, miRNAs that bind to Ago without interacting with target mRNAs are susceptible to degradation, whereas miRNAs that interact with target mRNAs are protected [48].

Furthermore, the raft-like regions of MVB are able to act as a target for miRNAs. Thus, independently of Ago, miRNAs binding either to the MVB raft-like regions, or to mRNAs themselves protects the miRNAs against degradation [54]. The high abundance of several passenger strands of miRNAs found in exosomes, may result from this protective property of the raft-like regions [52].

Exosomal miRNAs in viral infections

As mentioned above, the critical role miRNAs in most biological processes, such as differentiation, proliferation, development and tumorigenesis, has described by several studies [87,88]. miRNAs regulate the expression of genes via binding to the 3′UTR of the target mRNA, which leads to dysregulation of target gene expression [51]. A single miRNA has the ability to influence several genes within any individual cell, or even affect the gene expression in adjacent cells and distant cells by the export mechanism. The export of miRNAs is mediated through active transport via the nuclear exporter XPO5 [89]. Recent studies have shown that miRNA secretion is partially carried out by exosome-mediated transfer mechanisms [86]. The roles of exosomal miRNAs in the regulation of the intercellular communications that occur in viral infections have been established (Table 1) [90].

Exosomal miRNAs in infections with DNA viruses

Gibbings *et al.* reported that exosomal transportation was involved in the modulation of miRNA activity in HeLa cells [77]. In addition to the host miRNAs, viral miRNAs have also been detected in exosomes derived from virus-infected cells. For example, some studies have detected BamHI fragment A rightward transcript (BART) viral miRNAs (ebv-miR-BHRF1-1 and ebv-miR-BART3) in exosomes originating from nasopharyngeal carcinoma cells infected with EBV [95,101]. It was found that ebv-BART15-3p was able to stimulate apoptosis in cancer cells through targeting Baculoviral IAP BIRC6 and TAX1BP1 leading to increased chemosensitivity of gastric cancer cells [102,103]. The effect of exosomal ebv-BART15-3p on cancer cells has not yet been studied and requires further investigation.

In vitro studies showed that EBV can induce the expression of viral genes in neighboring noninfected cells by exosomal transmission of EBV-miRNAs. Pegtel *et al.* reported that exosomes play a critical role in the transfer of viral miRNAs from EBV-infected B cells to primary immature monocyte-derived DCs, resulting in repression of target genes [104]. The coculture model was used in this investigation to show that exosomes could transfer EBV miRNAs from EBV-infected cells to uninfected cells. The coculture approach indicated the transfer of EBV-miRNAs was more physiologically relevant compared with the use of purified exosomes alone.

Table 1. Exosomal miRNAs and viral infection.

Virus	miRNA	Expression	Method in the exosome	Sample	Ref.
Influenza	miR-483-3p	Up	Microfiltration + ultracentrifugation	Bronchoalveolar lavage fluid	[91]
HCV	miR-192	–	ExoQuick-TC	Cell culture (Huh-7)	[92]
HPV16	miR-222-3p	Up	Differential centrifugation + filtration	Cell culture (KSFM)	[93]
EBV	miR-155	UP	Exospin	Cell culture (human foreskin keratinocytes)	[94]
EBV	ebv-miR-BART3	–	Differential centrifugation + verified by nanosight and Western blot	Peripheral blood monocular cells	[95]
EBV	ebv-miR-BHRF1-1	–	Differential centrifugation + verified by nanosight and Western blot	Peripheral blood monocular cells	[95]
HIV	hsa-miR-29a	Up (strongly)	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-150	Up (strongly)	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-875	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-518f	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-1243	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-1274a	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-302c	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-872	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-636	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-30e	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-548a	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-454	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
HIV	hsa-miR-338	Up	Iodixanol gradient + ultracentrifugation	Cell culture (HIV-infected macrophage)	[96]
EV71	miR-146a	–	Exoquick	Serum	[97]
HSV-1	miR-H28	–	Life Technology	Cell culture (HEK293T and HEp-2)	[98]
HSV-1	miR-H29	–	Life Technology	Cell culture (HEK293T and HEp-2)	[98]
KSHV	miR-92a	–	Ultracentrifugation + microfiltration + ExoQuick-TC	Cell culture (BCBL-1)	[99]
KSHV	mir-10b-5p	–	Ultracentrifugation + microfiltration + ExoQuick-TC	Cell culture (BCBL-1)	[99]
KSHV	mir-143-3p	–	Ultracentrifugation + microfiltration + ExoQuick-TC	Cell culture (BCBL-1)	[99]
KSHV	miR-K12-8-3p	–	Ultracentrifugation + microfiltration + ExoQuick	Cell culture (BCBL-1)	[99]
KSHV	miR-K12-4-3p	–	Ultracentrifugation + microfiltration + ExoQuick-TC	Cell culture (BCBL-1)	[99]
KSHV	miR-K12-2-5p	–	Ultracentrifugation + microfiltration + ExoQuick-TC	Cell culture (BCBL-1)	[99]
KSHV	miR-17-92 cluster	Up	Filtration + ultracectrifugation + ExoQuick	*In vitro*	[100]
KSHV	KSHV-miRs	–	Filtration + ultracectrifugation + ExoQuick	*In vitro*	[100]

EBV: Epstein–Barr virus; EV71: Enterovirus 71; HCV: Hepatitis C virus; HPV: Human papillomavirus; KSFM: Keratinocyte-serum-free media; KSHV: Kaposi sarcoma-associated herpesvirus.

The Kaposi sarcoma-associated herpesvirus (KSHV or HHV8) modifies its microenvironment by transferring its miRNAs within secreted exosomes, from KSHV-infected lymphatic endothelial cells to adjacent cells. While neighboring cells remained uninfected, the KSHV miRNAs influence these cells by downregulating the expression levels of their target genes. These changes lead to a metabolic shift into aerobic glycolysis and to decreased mitochondrial biogenesis in primary lymphatic endothelial cells. This process reduced the sensitivity of exosome-recipient cells to KSHV, but increased their potential for angiogenesis and migration [13].

In a recent study, Honegger *et al.* examined the effect of human papillomavirus (HPV) oncogenes on the miRNA composition of exosomes. They reported that continuous expression of HPV E6 and E7 oncoproteins was linked to the downregulation of miR-21-5p, and upregulation of miR-20a-5p, miR-423-3p, miR-92a-3p, miR-378a-3p, miR-7-5p and let-7d-5p in exosomes released from HeLa cells [12]. Some of these miRNAs may be involved in cancer-associated growth and progression [105]. For example, let-7d-5p (which is a member of the let-7d family) has antiapoptotic activity. A recent study reported that the expression of let-7d-5p was significantly increased during breast cancer progression [106]. miR-423-3p has been found to be involved in cell growth, and promotes the G1/S cell-cycle transition [107]. These findings suggest that the endogenous expression of HPV E6/E7 in cancer cells is associated with alteration of the miRNAs within exosomes, partially explaining the antiapoptotic or

pro-proliferative effects [12]. Recent studies have found that miR-222-3p and miR-320a are expressed in exosomes originating from primary human foreskin keratinocytes (HFKs). Interestingly, miR-222-3p and miR-320a were respectively downregulated and upregulated inside HPV16 E6/E7-infected HFKs, while these miRNAs were upregulated and downregulated in the bexosomes that were released from infected cells [93,108]. These data suggest that miRNAs can be selectively packaged into exosomes. Harden and Munger examined the effect of exosomal miRNAs secreted from HFKs on the processes of necrosis, apoptosis and cell survival. They suggested that the expression of HPV16 E6 and E7 proteins in transfected HFKs inhibited necrosis and apoptosis in adjacent normal cells through the effect of exosomal miRNAs [108]. In HPV infection, the HPV genome integrates into the host genome. This integration results in the complete or partial loss of the *E1* and *E2* genes, which modulate the activity of the viral oncoproteins E6 and E7. However, no investigations have yet been carried out to compare the expression profiles of exosomal miRNAs in HPV-integrated cells in comparison with nonintegrated HPV DNA.

BK viral nephropathy (BKVN) is caused by the BK polyoma virus (BKV), and leads to graft failure and deterioration of transplanted kidneys in recipients receiving immunosuppression [109]. Molecular surveillance utilizing BK viral load assays in urine samples and plasma has led to the number of proven BKVN biopsies declining (1–10% rate of infection) [110]. Nevertheless a considerable proportion of infected kidney transplant recipients suffer from graft injury with a 10–80% rate of graft loss [109]. It has been recently reported that BKV-encoded miRNAs can be detected in the urine and blood of BKV-infected patients [111].

Kim *et al.* assessed the expression of BK viral miRNAs in exosomes in the urine during virus replication, and the possible diagnostic property to identify BKVN in recipients of kidney transplants [112]. Their findings reported that high concentrations of bkv-miR-B1-3p and bkv-miR-B1-5p were observed in all the BKVN patients. Meanwhile, plasma and urinary BK viral load assays displayed a false negative in three and one cases, respectively, among 13 affected patients. The receiver-operating characteristic analysis for bkv-miR-B1-5p/miR-16 and bkv-miR-B1-5p markers showed a good discriminative power for BKVN, with area under the curve values of 0.985 and 0.989, respectively. Their results suggested that detection of exosomal bkv-miR-B1-5p/miR-16 and bkv-miR-B1-5p in urine could be biomarkers for BKVN diagnosis [112]. The fact that BKV can also be detected in the urine from healthy subjects [113–115] suggests that urinary exosomal BKV miRNAs should be compared between normal individuals and BKNV patients.

Bayer *et al.* [116] found that infection with varicella zoster virus could be attenuated in cells that had been pre-exposed to exosome-packaged miRNA clusters from chromosome 19. Delorme-Axford *et al.* [117] examined the effect of miRNAs within trophoblast-derived exosomes on antiviral responses, and found that miRNAs attenuated the replication of vaccinia virus in recipient cells by induction of autophagy.

Marek's disease (MD) is a complex lymphoproliferative disease of chickens caused by the 'Marek's disease virus' (MDV), which is characterized by T-cell lymphoma, immunosuppression and general paralysis [118,119]. MD is generally controlled through administration of vaccines, which lead to protection against the formation of lymphoma, but does not prevent against superinfection by MDV field strains. In spite of the demonstration of cell-mediated and humoral immune responses elicited by the vaccine, the mechanisms that elicit protection remain obscure. Neerukonda *et al.* investigated the contents of serum exosomes to evaluate their potential role as indicators of systemic immunity and risk of tumor formation [120]. They studied the protein and RNA contents of serum exosomes from unvaccinated tumor-bearing chickens (TEX) and from CVI988 (Rispens)-vaccinated chickens (VEX) using mass spectrometry and deep sequencing. In comparison to TEX, higher amounts of tumor-suppressor miRNAs were found in VEX, based on bioinformatic analysis of miRNAs. On the other hand, oncomiRs (e.g., miRs 106a-363) as well as miRNA MDV clusters were more abundant in TEX in comparison with VEX. mRNAs that mapped to the unique long flanking repeats of MDV (IR_L/TR_L) were found in TEX, while mRNAs that mapped to the whole genome of MDV were found in VEX. These findings suggested that long-term vaccination responses could involve the systemic transfer of viral mRNAs to antigen presenting cells. Proteomic analysis of the exosomes found biomarkers that could distinguish TEX from VEX [120].

Exosomal miRNAs in infections with RNA viruses

Exosomes derived from primary alveolar macrophages (human monocytic leukemia THP-1 cells) infected with HIV-1 contain the viral miRNAs, vmiR-TAR, vmiR99 and vmiR88. Moreover, these miRNAs have also been detected in exosomes isolated from HIV-1-infected patient sera. vmiR99 and vmiR88 were demonstrated to stimulate signaling pathways in macrophages resulting in the robust secretion of TNFα by activation of endosomal TLR8 [121].

It has been observed that expression of the HIV Nef (negative regulatory factor) in macrophage-like cells could modulate the exosomal miRNA composition, and affect specific mechanisms for recruitment and maintenance of specific miRNAs in exosomes or cells. Astrocytes that were exposed to a combination of morphine and HIV Tat protein, released exosomes with increased levels of miR-29b [122]. After exposure of human neurons to exosomes enriched with miR-29b, expression of PDGF B was suppressed, and the viability of neurons was correspondingly reduced [122]. Argonaute 2 (Ago-2) is an essential protein component of the RISC that mediates miRNA-targeted gene inhibition. It was observed that exosomes derived from human T-cell line (C8166-45 cells) infected with human T-lymphotropic virus (HTLV)-1 could manipulate translation of mRNA in recipient cells, suggesting that the HTLV-1 proteins interfere with Ago-2 function [123]. In mice infected with various influenza virus strains, miR-483-3p was present at an elevated level in exosomes, which were isolated from bronchoalveolar lavage fluid. RNF5 and CD81 are regulators of the RIG-I signaling pathway, and both of them are miR-483-3p targets in MLE-12 cells. Maemura *et al.* reported that transfection of miR-483-3p induced expression of proinflammatory cytokine and type I interferon, in MLE-12 (murine lung epithelial) cells infected with influenza virus infection. Taken together, these studies showed that bronchoalveolar lavage fluid exosomal miRNAs could mediate the inflammatory and antiviral responses after influenza infection [91].

miRNAs act as key mediators in the liver disease induced by hepatitis C virus (HCV) [124]. The pattern of miRNA expression is altered in hepatic stellate cells (HSCs) infected with HCV. The HCV core protein leads to increased miR-192 levels in hepatocytes, and miR-192 can stimulate transcription of TGF-b1 by targeting ZEB1 [125]. Kim *et al.* identified miR-192 in the exosomes released from the hepatocyte cell line (Huh-7) infected with HCV. Exosomes can stimulate the transdifferentiation of the LX-2 HSCs line, and trigger the transformation of HSCs into myofibroblasts via the action of TGF-β1 [92].

Exosomes are also significant mediators of the interaction between tumors and their environment. Cancer-derived exosomes can effectively disrupt the integrity of the vascular endothelial barrier and disrupt tight junctions to promote tumor cell metastasis [126]. Additionally, exosomes secreted by lung cancer cells can regulate their migration by IL-10 and TGF-β signaling [127]. Moreover, exosomes released from mesenchymal stem cells could inhibit HCC cell proliferation. Mast cell-originated exosomes activate T and B lymphocytes to play a proinflammatory role [128]. The tumor-suppressor miR-490 plays a significant role in the invasion, migration and growth of breast, ovarian and colon cancers [129–131]. Furthermore, miR-490 regulates the metastasis and proliferation of HCC cells via targeting Endoplasmic Reticulum-Golgi Intermediate Compartment Protein 3 [132]. On average, 70% of liver cancer cells express a high level of EGFR, and activated EGFR has an important role in migration and angiogenesis [133,134]. EGFR inhibition is a potential therapeutic approach for cancer treatment. Xiong *et al.* found that the E2 protein of HCV increased the level of miR-490 in exosomes derived from mast cells, and when transferred to recipient HCC cells, eventually decreased the activity of the EGFR/AKT/ERK1/2 signaling pathway and inhibited HCC cell migration [126].

Interferons (IFNs) are cytokines produced by cells as a defensive response against viruses [135]. Some viruses, which indirectly interfere with TRAF6 and RAK1, two important factors involved in IFN production and TLR signaling, are able to escape the antiviral effects of IFN. It has been shown that infection with enterovirus 71 (EV71) can upregulate miR-146a that facilitates the pathogenicity of the virus via suppression of STAT1, IRAK1 and TRAF6 [136]. In order to validate the effect of miR-146a, a mouse-adapted version of the virus mEV71 was established from human EV71. The mice were orally infected with mEV71, and pulmonary edema, mortality, loss of body weight and paralysis were similar to those symptoms seen in humans. However, some pathological findings in the EV71-infected mice were not identical to human clinical findings. Of note, heart failure, pulmonary edema and limb paralysis symptoms that have been observed in humans may be due to the lytic EV71 replication in the CNS, while the replication of mEV71 may occur in a wider range of organs in mEV71-infected mice. Therefore, whether EV71-infected animals can be used to discover novel antiviral therapies for clinical trials, as well as to understand the underlying pathological mechanisms requires further evaluation [136]. Fu *et al.* showed that exosomes released from EV71-infected human monocytic leukemia (THP-1) cells and human colon cancer (HT-29) cells selectively contained high levels of miR-146a. These exosomes could be functionally transferred into recipient cells and facilitate the replication of the virus by suppressing IFN responses [97]. Moreover, because of the similarity between exosomes and EV71 virions in terms of their sedimentation velocity and buoyant density [137,138], the sucrose gradient isolation technique and traditional ultracentrifugation is not guaranteed to isolate pure exosomes that are free from cellular and viral contamination. Therefore, a CD63 immunomagnetic bead isolation technique was optimized for purifying EV71-related exosomes, without any carryover of free virus, and used to confirm that

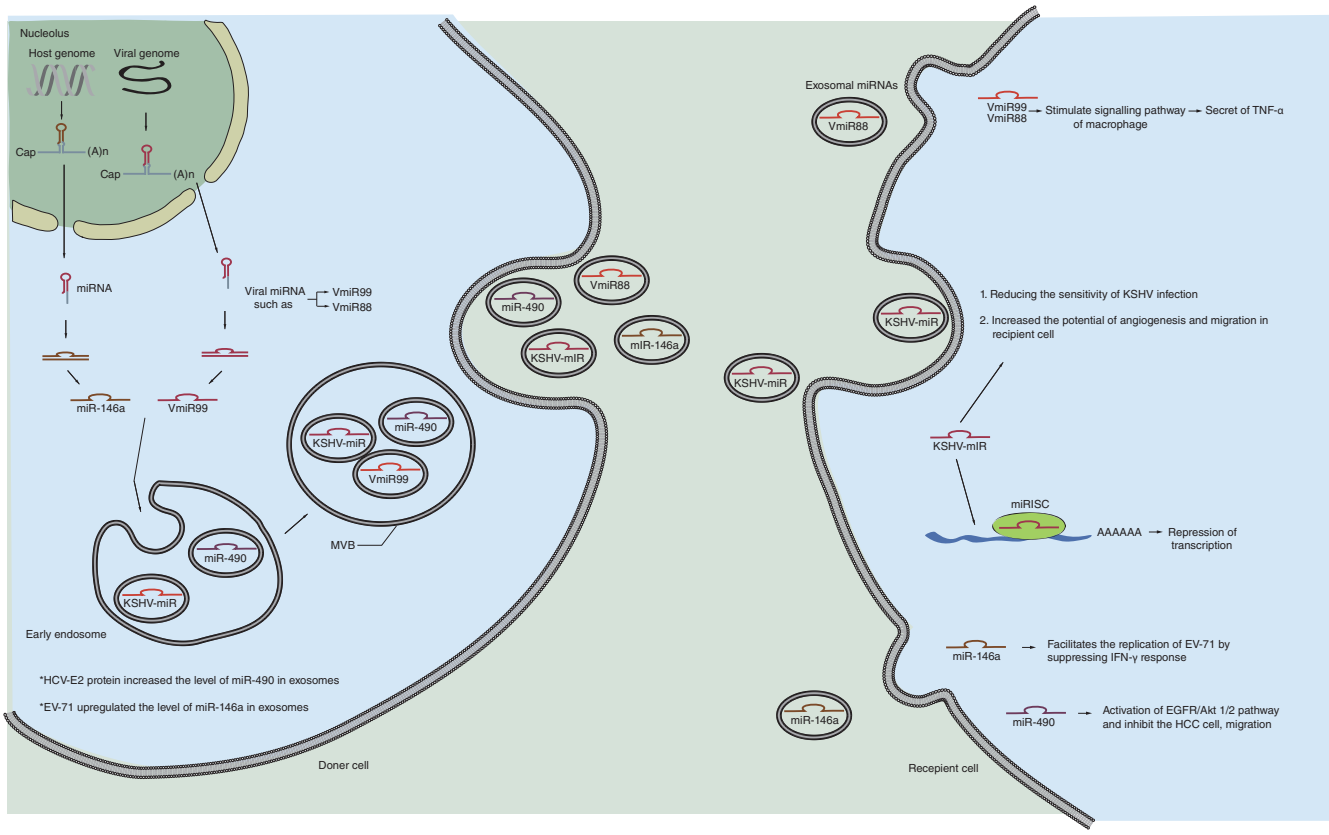

Figure 3. Exosomal miRNA biogenesis in viral infection. Certain viral proteins such as ECV-E2 and EV71 can change the miRNAs in exosomes. On the other hand, certain miRNAs can affect host response to viruses and either stimulate or inhibit infection.

the EV71 RNA was indeed intra-exosomal. EV71 infection was recently revealed to be an infection that could be transmitted through exosomes [139]. Although the viral proteins were detected in the exosomes isolated from RD cells infected by EV71 [139], they were not found in the exosomes in all the situations that were studied. This difference may be because the two-step purification approach is superior to ultracentrifugation alone, and can avoid isolating exosomes contaminated with multiple foreign proteins [140,141].

Bayer *et al.* [116] found that the infection of cells with rubella, other togaviruses and HIV-1 could be attenuated if the cells had been pre-exposed to exosome-packaged miRNA clusters from chromosome 19. Delorme-Axford *et al.* [117] demonstrated that miRNAs contained within trophoblast-derived exosomes showed antiviral activity, and attenuated viral replication in recipient cells by the induction of autophagy. They found that human miR-512-3p, miR-516b-5p and miR-517-3p, which were packaged within primary human trophoblast (PHT)-derived exosomes conferred viral resistance to recipient cells. They showed that exposure of PHT recipient cells and non-PHT recipient cells including HeLa, U2OS and human foreskin fibroblasts for 24 h before infection, to miRNAs isolated from naive PHT cells decreased the replication of several viruses including coxsackievirus B, vesicular stomatitis virus and HCV [117].

Exosomal miRNAs have been consistently detected in saliva, blood, urine, breast milk and synovial fluid. The composition and amount of exosomal miRNAs are different between virally infected and noninfected cells. Therefore, exosomal miRNAs may play a potential role as noninvasive biomarkers for diagnosis and monitoring of viral diseases.

Taken together, the above-mentioned evidence suggests that exosomal miRNAs originating from virally infected cells, can alter the expression of target genes in recipient cells and therefore they could be used to control viral diseases. Figure 3 illustrates the biogenesis of exosomal miRNAs in viral infections.

The functions of exosomes in viral infections

As previously mentioned, exosomes isolated from virus-infected cells contain many viral components such as proteins, mRNAs, genomic RNA and miRNAs. Exosomes secreted from HIV-infected cells contain the viral transactivation response element and lead to elevated HIV replication in the recipient cells by downregulating apoptosis [142]. Esser *et al.* reported that MHC class II, CD86 (B7-2) and CD45 molecules were all contained in exosomes originating from immune cells (H9, CEMX174/T1, CEMX174/T2 and TBLCL-CD4) infected with HIV-1. These mediators are likely to be involved in silencing the immune response, thus favoring virus replication [143]. Because exosomes play a significant role in tumor pathogenesis, it is not surprising that many oncogenic viruses from several DNA virus families, and different RNA viruses such as flaviviruses and retroviruses, have been shown to interfere with the normal exosomal communication function. Some of the HPV subtypes (HPV16 and 18) are associated with cervical malignancy. Several studies have reported that exosomes released from cells infected with oncogenic HPV subtypes were enriched with miRNAs that controlled cell apoptosis and proliferation. The Tax protein of HTLV-1, which is an important factor for transformation and proliferation of $CD4^+$ T lymphocytes, was found to be incorporated in exosomes derived from HTLV-infected cells. Overall, exosomes from virus-infected cells and from cancer cells tend to promote the progression of viral infections or malignancy by adversely affecting the function of normal recipient host cells.

Conclusion & Future perspective

Viral infections are known to be a very important cause of respiratory tract disease in the outpatient setting, but much less common in the intensive care unit. It has been shown that viral infections exert their pathological impact though activation/inhibition of a wide range of cellular and molecular mechanisms.

Exosomes are a new class of mediators involved in intercellular signaling, which have been implicated in several different diseases and pathological conditions, including viral-infections. Increasing evidence has suggested that exosomes can target their cargos to recipient cells, and are able to change the behavior of these cells. A variety of biological molecules (RNA, DNA, proteins and lipids) can be transferred by exosomes. miRNAs are epigenetic regulators that exert their effects via downregulating gene expression, and also affecting a range of cellular and molecular pathways. The transfer of these miRNAs into various recipient cells by means of exosomes is associated with an alteration in different aspects of cellular behavior. The ability of exosomes to transport miRNAs to adjacent cells or even to distant cells may explain some novel modulatory mechanisms, which are characteristic of viral infections. Many studies on exosomes secreted from virus-infected cells have suggested that modulation of the exosomal miRNA composition during viral infection is a regulated process, in which multiple mechanisms for production or maintenance of specific miRNAs are involved. Therefore, it is likely that manipulation of exosomal miRNAs by specific viruses contributes to the viral escape from the host immune response, promotes survival of infected cells, allows spread of infection to noninfected cells and maybe play a role in the maintenance of latent and persistent viral infections.

It seems that further study of exosomal miRNAs, especially as they are related to viral infections, could open new horizons in the development of new diagnostic biomarkers and therapeutic approaches for the treatment of patients who are infected by viruses. In addition, viral miRNAs and host-related miRNAs are also involved in various types of viral latency. Hence, the characterization of miRNAs (derived from the host cells and also from the viral genome) contained in exosomes is important to fully understand the viral life cycle. Various reports have assessed the host and/or viral miRNA expression profiles in viral-associated tumor tissues and in viral-infected cell lines by real-time quantitative PCR, microarrays and next-generation sequencing assays. Little is understood about host and viral miRNA expression profiles in exosomes that are derived from viral-infected cells. Hence, more work is needed to gain a precise understanding of the cellular and molecular pathways, which are either activated or inhibited by host miRNAs and/or viral miRNAs.

Financial & competing interests disclosure

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Executive summary

Biogenesis of exosomes

- Exosomes are secreted by direct budding from the plasma membrane of cells, and can be isolated from cultured cells and also from human body fluids.
- The biogenesis of exosomes is mediated via the endosomal sorting complex transport machinery.
- Cells expressing Epstein–Barr virus (EBV)-encoded LMP1 released more exosomes in comparison with control cells, and involved CD63.

MiRNA biogenesis

- The majority of miRNAs are transcribed as long primary miRNAs. This process is regulated by the MEDIATOR complex.
- One guide strand of miRNA duplex, usually an A/G-rich strand with a 5′-U initiation site binds to Argonaute protein within the RNA-induced silencing complex.
- The miRNA guides RNA-induced silencing complex to bind to complementary target mRNA, and represses its translation via binding to the 3′UTR.
- There may also be lipid-induced loading of RNA into exosomes.
- Spontaneous inward budding of the raft-like areas of the multivesicular body membrane creates intraluminal vesicles, enriched in phosphatidylcholine, saturated fatty acids, glycosphingolipids, sphingomyelin and cholesterol.
- The binding of RNAs to the membrane is mediated via sphingosine, lipid structures (especially lipid rafts) and hydrophobic modifications. Some specific nucleotide sequences show increased affinity to phospholipid bilayers.
- RNAs are selected based on their affinity to the raft-like areas in the multivesicular body membrane to be incorporated into intraluminal vesicles, but the budding process is independent of RNA binding.

Exosomal miRNAs in infections with DNA viruses

- Exosomal transportation is involved in the modulation of miRNA activity in cells.
- Host miRNAs and viral miRNAs have also been detected in exosomes derived from virus-infected cells.
- BamHI fragment A rightward transcript (BART) viral miRNAs (ebv-miR-BHRF1-1 and ebv-miR-BART3) have been detected in exosomes originating from nasopharyngeal carcinoma cells-infected with EBV.
- EBV can induce the expression of viral genes in neighboring noninfected cells by exosomal transmission of EBV-miRNAs.

Exosomal miRNAs in infections with RNA viruses

- Exosomes derived from primary alveolar macrophages (human monocytic leukemia THP-1 cells) infected with HIV-1 carried viral miRNAs, vmiR-TAR, vmiR99 and vmiR88. These miRNAs have also been detected in exosomes isolated from HIV-1-infected patient sera.
- The expression of the HIV Nef (negative regulatory factor) in macrophage-like cells can modulate exosomal miRNA composition, and affects the mechanisms for recruitment and maintenance of specific miRNAs in exosomes or cells.
- Exosomes released from enterovirus 71-infected human monocytic leukemia (THP-1) cells and human colon cancer (HT-29) cells both contained high levels of miR-146a.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Zhang J, Li S, Li L *et al.* Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics, Proteomics Bioinformatics* 13(1), 17–24 (2015).

• **Covers the underlying mechanisms of exosome biogenesis and miRNA trafficking.**

2. Pelchen-Matthews A, Raposo G, Marsh M. Endosomes, exosomes and Trojan viruses. *Trends Microbiol.* 12(7), 310–316 (2004).

3. Hu G, Drescher KM, Chen X. Exosomal miRNAs: biological properties and therapeutic potential. *Front. Genet.* 3, 56 (2012).

• **Discusses possible therapeutic roles of exosomal miRNAs.**

4. Trobaugh DW, Klimstra WB. MicroRNA regulation of RNA virus replication and pathogenesis. *Trends Mol. Med.* 23(1), 80–93 (2017).

- **Discusses the role of miRNAs in viral pathogenesis.**

5. Wang L, Li G, Yao ZQ, Moorman JP, Ning S. MicroRNA regulation of viral immunity, latency, and carcinogenesis of selected tumor viruses and HIV. *Rev. Med. Virol.* 25(5), 320–341 (2015).
6. Su Z, Yang Z, Xu Y, Chen Y, Yu Q. MicroRNAs in apoptosis, autophagy and necroptosis. *Oncotarget* 6(11), 8474 (2015).
7. Plaisance-Bonstaff K, Renne R. Viral miRNAs. In: *Antiviral RNAi*. Springer, 43–66 (2011).
8. Naqvi AR, Shango J, Seal A, Shukla D, Nares S. Viral miRNAs alter host cell miRNA profiles and modulate innate immune responses. *Front. Immunol.* 9, 433 (2018).
9. Bhome R, Del Vecchio F, Lee G-H *et al.* Exosomal microRNAs (exomiRs): small molecules with a big role in cancer. *Cancer Lett.* 420, 228–235 (2018).
10. Wiertz EJ, Mukherjee S, Ploegh HL. Viruses use stealth technology to escape from the host immune system. *Mol. Med. Today* 3(3), 116–123 (1997).
11. Hilleman MR. Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. *Proc. Natl Acad. Sci. USA* 101(Suppl. 2), 14560–14566 (2004).
12. Honegger A, Schilling D, Bastian S *et al.* Dependence of intracellular and exosomal microRNAs on viral E6/E7 oncogene expression in HPV-positive tumor cells. *PLoS Pathog.* 11(3), e1004712 (2015).
13. Yogev O, Henderson S, Hayes MJ *et al.* Herpesviruses shape tumour microenvironment through exosomal transfer of viral microRNAs. *PLoS Pathog.* 13(8), e1006524 (2017).
14. Crenshaw BJ, Sims B, Matthews QL. Biological function of exosomes as diagnostic markers and therapeutic delivery vehicles in carcinogenesis and infectious diseases. In: Muhammad Akhyar Farrukh (Ed). *Nanomedicines.* IntechOpen, London, UK (2018).
15. Yáñez-Mó M, Siljander PR-M, Andreu Z *et al.* Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* 4(1), 27066 (2015).
16. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.* 200(4), 373–383 (2013).
17. Février B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr. Opin. Cell Biol.* 16(4), 415–421 (2004).
18. Colombo M, Moita C, Van Niel G *et al.* Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *J. Cell Sci.* 126(24), 5553–5565 (2013).
19. Deng Y, Wang CC, Choy KW *et al.* Therapeutic potentials of gene silencing by RNA interference: principles, challenges, and new strategies. *Gene* 538(2), 217–227 (2014).
20. Kubowicz P, Zelaszczyk D, Pekala E. RNAi in clinical studies. *Curr. Med. Chem.* 20(14), 1801–1816 (2013).
21. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. *Trends Mol. Med.* 20(8), 460–469 (2014).
22. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat. Rev. Genet.* 12(2), 99 (2011).
23. Camilleri ET, Gustafson MP, Dudakovic A *et al.* Identification and validation of multiple cell surface markers of clinical-grade adipose-derived mesenchymal stromal cells as novel release criteria for good manufacturing practice-compliant production. *Stem Cell Res. Ther.* 7(1), 107 (2016).
24. Ghaneialvar H, Soltani L, Rahmani HR, Lotfi AS, Soleimani M. Characterization and classification of mesenchymal stem cells in several species using surface markers for cell therapy purposes. *Indian J. Clin. Biochem.* 33(1), 46–52 (2018).
25. Bashiri H, Amiri F, Hosseini A *et al.* Dual preconditioning: a novel strategy to withstand mesenchymal stem cells against harsh microenvironments. *Adv. Pharm. Bull.* 8(3), 465 (2018).
26. Hosseini S, Taghiyar L, Safari F, Eslaminejad MB. Regenerative medicine applications of mesenchymal stem cells. In: *Cell Biology and Translational Medicine, Volume 2*. Springer, 115–141 (2018).
27. Rohban R, Pieber TR. Mesenchymal stem and progenitor cells in regeneration: tissue specificity and regenerative potential. *Stem Cell. Int.* 2017, 5173732 (2017).
28. Rodini CO, Da Silva PBG, Assoni AF, Carvalho VM, Okamoto OK. Mesenchymal stem cells enhance tumorigenic properties of human glioblastoma through independent cell-cell communication mechanisms. *Oncotarget* 9(37), 24766 (2018).
29. Cheng X, Zhang G, Zhang L *et al.* Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration. *J. Cell. Mol. Med.* 22(1), 261–276 (2018).
30. Roushandeh AM, Bahadori M, Roudkenar MH. Mesenchymal stem cell-based therapy as a new horizon for kidney injuries. *Arch. Med. Res.* 48(2), 133–146 (2017).
31. Amiri F, Jahanian-Najafabadi A, Roudkenar MH. *In vitro* augmentation of mesenchymal stem cells viability in stressful microenvironments. *Cell Stress Chaperones* 20(2), 237–251 (2015).
32. Zhaleh F, Amiri F, Mohammadzadeh-Vardin M *et al.* Nuclear factor erythroid-2 related factor 2 overexpressed mesenchymal stem cells transplantation, improves renal function, decreases injuries markers and increases repair markers in glycerol-induced Acute kidney injury rats. *Iran. J. Basic Med. Sci.* 19(3), 323 (2016).

33. Zhou J, Tan X, Tan Y, Li Q, Ma J, Wang G. Mesenchymal stem cell derived exosomes in cancer progression, metastasis and drug delivery: a comprehensive review. *J. Cancer* 9(17), 3129 (2018).
34. Qiu G, Zheng G, Ge M *et al.* Mesenchymal stem cell-derived extracellular vesicles affect disease outcomes via transfer of microRNAs. *Stem Cell Res. Ther.* 9(1), 320 (2018).
35. Yeo RWY, Lai RC, Zhang B *et al.* Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. *Adv. Drug Delivery Rev.* 65(3), 336–341 (2013).
36. Nakamura Y, Miyaki S, Ishitobi H *et al.* Mesenchymal-stem-cell-derived exosomes accelerate skeletal muscle regeneration. *FEBS Lett.* 589(11), 1257–1265 (2015).
37. Bai L, Shao H, Wang H *et al.* Effects of mesenchymal stem cell-derived exosomes on experimental autoimmune uveitis. *Sci. Rep.* 7(1), 4323 (2017).
38. Sun L, Li D, Song K *et al.* Exosomes derived from human umbilical cord mesenchymal stem cells protect against cisplatin-induced ovarian granulosa cell stress and apoptosis *in vitro*. *Sci. Rep.* 7(1), 2552 (2017).
39. Pachler K, Ketterl N, Desgeorges A *et al.* An *in vitro* potency assay for monitoring the immunomodulatory potential of stromal cell-derived extracellular vesicles. *Int. J. Mol. Sci.* 18(7), 1413 (2017).
40. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res.* 65(14), 6029–6033 (2005).
41. Reza-Zaldivar EE, Hernandez-Sapiéns MA, Minjarez B, Gutierrez-Mercado YK, Marquez-Aguirre AL, Canales-Aguirre AA. Potential effects of MSC-derived exosomes in neuroplasticity in Alzheimer's disease. *Front. Cell. Neurosci.* 12(317) (2018).
42. Xin H, Wang F, Li Y *et al.* Secondary release of exosomes from astrocytes contributes to the increase in neural plasticity and improvement of functional recovery after stroke in rats treated with exosomes harvested from microRNA 133b-overexpressing multipotent mesenchymal stromal cells. *Cell Transplant.* 26(2), 243–257 (2017).
43. Caruso S, Poon IK. Apoptotic cell-derived extracellular vesicles: more than just debris. *Front. Immunol.* 9, 1486 (2018).
44. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. *J. Cereb. Blood Flow Metab.* 33(11), 1711–1715 (2013).
45. Janas MM, Khaled M, Schubert S *et al.* Feed-forward microprocessing and splicing activities at a microRNA–containing intron. *PLoS Genet.* 7(10), e1002330 (2011).
46. Achkar NP, Cambiagno DA, Manavella PA. miRNA biogenesis: a dynamic pathway. *Trends Plant Sci.* 21(12), 1034–1044 (2016).
47. Siomi H, Siomi MC. Posttranscriptional regulation of microRNA biogenesis in animals. *Mol. Cell* 38(3), 323–332 (2010).
48. Meijer HA, Smith EM, Bushell M. Regulation of miRNA strand selection: follow the leader? *Biochem. Soc. Trans.* 42(4), 1135–1140 (2014).
49. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat. Rev. Mol. Cell Biol.* 10(2), 126 (2009).
50. Okada C, Yamashita E, Lee SJ *et al.* A high-resolution structure of the pre-microRNA nuclear export machinery. *Science* 326(5957), 1275–1279 (2009).
51. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat. Rev. Genet.* 5(7), 522 (2004).
52. Bang C, Batkai S, Dangwal S *et al.* Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *J. Clin. Invest.* 124(5), 2136–2146 (2014).
53. Koppers-Lalic D, Hackenberg M, Bijnsdorp IV *et al.* Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. *Cell Rep.* 8(6), 1649–1658 (2014).
54. Janas T, Janas MM, Sapon K, Janas T. Mechanisms of RNA loading into exosomes. *FEBS Lett.* 589(13), 1391–1398 (2015).
55. Janas T, Janas T. The selection of aptamers specific for membrane molecular targets. *Cell Mol. Biol. Lett.* 16(1), 25–39 (2011).
56. Janas T, Janas T, Yarus M. Human tRNA(Sec) associates with HeLa membranes, cell lipid liposomes, and synthetic lipid bilayers. *RNA* 18(12), 2260–2268 (2012).
57. Janas T, Yarus M. Visualization of membrane RNAs. *RNA* 9(11), 1353–1361 (2003).
58. Vlassov A, Khvorova A, Yarus M. Binding and disruption of phospholipid bilayers by supramolecular RNA complexes. *Proc. Natl Acad. Sci. USA* 98(14), 7706–7711 (2001).
59. Khvorova A, Kwak YG, Tamkun M, Majerfeld I, Yarus M. RNAs that bind and change the permeability of phospholipid membranes. *Proc. Natl Acad. Sci. USA* 96(19), 10649–10654 (1999).

- **Raft-like areas on the cytoplasmic surface of the multivesicular body (MVB) membrane are the regions for loading different RNAs into exosomes.**

60. Janas T, Janas T, Yarus M. A membrane transporter for tryptophan composed of RNA. *RNA* 10(10), 1541–1549 (2004).
61. Tani M, Hannun YA. Neutral sphingomyelinase 2 is palmitoylated on multiple cysteine residues. Role of palmitoylation in subcellular localization. *J. Biol. Chem.* 282(13), 10047–10056 (2007).

62. Wu BX, Clarke CJ, Matmati N, Montefusco D, Bartke N, Hannun YA. Identification of novel anionic phospholipid binding domains in neutral sphingomyelinase 2 with selective binding preference. *J. Biol. Chem.* 286(25), 22362–22371 (2011).
63. Rappa G, Mercapide J, Anzanello F, Pope RM, Lorico A. Biochemical and biological characterization of exosomes containing prominin-1/CD133. *Mol. Cancer* 12(1), 62 (2013).
64. Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell–cell communication and various pathophysiologies. *Biochim. Biophys. Acta* 1841(1), 108–120 (2014).
65. Kajimoto T, Okada T, Miya S, Zhang L, Nakamura S-I. Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. *Nat. Commun.* 4, 2712 (2013).
66. Gulbins E, Kolesnick R. Raft ceramide in molecular medicine. *Oncogene* 22(45), 7070 (2003).
67. Chiantia S, Kahya N, Ries J, Schwille P. Effects of ceramide on liquid-ordered domains investigated by simultaneous AFM and FCS. *Biophys. J.* 90(12), 4500–4508 (2006).
68. And I, Johnston LJ. Ceramide promotes restructuring of model raft membranes. *Langmuir* 22(26), 11284–11289 (2006).
69. Nurminen TA, Holopainen JM, Zhao H, Kinnunen PK. Observation of topical catalysis by sphingomyelinase coupled to microspheres. *J. Am. Chem. Soc.* 124(41), 12129–12134 (2002).
70. Trajkovic K, Hsu C, Chiantia S *et al.* Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 319(5867), 1244–1247 (2008).

• **Lipid-mediated inward budding of the raft-like area of the MVB membrane is a mechanism to load different RNAs into exosomes.**

71. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J. Biol. Chem.* 285(23), 17442–17452 (2010).
72. Llorente A, Skotland T, Sylvänne T *et al.* Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. *Biochim. Biophys. Acta* 1831(7), 1302–1309 (2013).
73. Balestrieri B, Hsu VW, Gilbert H *et al.* Group V secretory phospholipase A2 translocates to the phagosome after zymosan stimulation of mouse peritoneal macrophages and regulates phagocytosis. *J. Biol. Chem.* 281(10), 6691–6698 (2006).
74. Prinetti A, Chigorno V, Prioni S *et al.* Changes in the lipid turnover, composition, and organization, as sphingolipid-enriched membrane domains, in rat cerebellar granule cells developing *in vitro*. *J. Biol. Chem.* 276(24), 21136–21145 (2001).
75. Sonnino S, Prinetti A, Mauri L, Chigorno V, Tettamanti G. Dynamic and structural properties of sphingolipids as driving forces for the formation of membrane domains. *Chem. Rev.* 106(6), 2111–2125 (2006).
76. De Gassart A, Géminard C, Février B, Raposo G, Vidal M. Lipid raft-associated protein sorting in exosomes. *Blood* 102(13), 4336–4344 (2003).
77. Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat. Cell Biol.* 11(9), 1143 (2009).

• **RNA binding to the raft-like areas of the MVB membrane is a mechanism for loading different RNAs into exosomes.**

78. Hullin-Matsuda F, Taguchi T, Greimel P, Kobayashi T. Lipid compartmentalization in the endosome system. Presented at: *Seminars in Cell & Developmental Biology* 31, 48–56 (2014).
79. Janas T, Janas T, Yarus M. Specific RNA binding to ordered phospholipid bilayers. *Nucleic Acids Res.* 34(7), 2128–2136 (2006).
80. Carayon K, Chaoui K, Ronzier E *et al.* Proteolipidic composition of exosomes changes during reticulocyte maturation. *J. Biol. Chem.* 286(39), 34426–34439 (2011).
81. Batagov AO, Kurochkin IV. Exosomes secreted by human cells transport largely mRNA fragments that are enriched in the 3'-untranslated regions. *Biol. Direct* 8, 12 (2013).
82. Kajimoto T, Okada T, Miya S, Zhang L, Nakamura S. Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. *Nat. Commun.* 4, 2712 (2013).
83. Batagov AO, Kuznetsov VA, Kurochkin IV. Identification of nucleotide patterns enriched in secreted RNAs as putative *cis*-acting elements targeting them to exosome nano-vesicles. *BMC Genom.* 12(Suppl. 3), S18 (2011).
84. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F *et al.* Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat. Commun.* 4, 2980 (2013).
85. Janas T, Janas T, Yarus M. Specific RNA binding to ordered phospholipid bilayers. *Nucleic Acids Res.* 34(7), 2128–2136 (2006).
86. Janas MM, Wang B, Harris AS *et al.* Alternative RISC assembly: binding and repression of microRNA-mRNA duplexes by human Ago proteins. *RNA* 18(11), 2041–2055 (2012).
87. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 136(2), 215–233 (2009).

•• **Shows that a variety of exosomal miRNAs are involved in viral infections.**

88. Guarnieri DJ, Dileone RJ. MicroRNAs: a new class of gene regulators. *Ann. Med.* 40(3), 197–208 (2008).
89. Stoorvogel W. Functional transfer of microRNA by exosomes. *Blood* 119(3), 646–648 (2012).

90. Boon RA, Vickers KC. Intercellular transport of microRNAs. *Arterioscler. Thromb. Vasc. Biol.* 33(2), 186–192 (2013).

91. Maemura T, Fukuyama S, Sugita Y *et al.* Lung-derived exosomal miR-483-3p regulates the innate immune response to influenza virus infection. *J. Infect. Dis.* 217(9), 1372–1382 (2018).

92. Kim JH, Lee CH, Lee S-W. Exosomal transmission of microRNA from HCV replicating cells stimulates transdifferentiation in hepatic stellate cells. *Mol. Ther. Nucleic Acids* 14, 483–497 (2019).

93. Chiantore MV, Mangino G, Iuliano M *et al.* Human papillomavirus E6 and E7 oncoproteins affect the expression of cancer-related microRNAs: additional evidence in HPV-induced tumorigenesis. *J. Cancer Res. Clin. Oncol.* 142(8), 1751–1763 (2016).

94. Yoon C, Kim J, Park G *et al.* Delivery of miR-155 to retinal pigment epithelial cells mediated by Burkitt's lymphoma exosomes. *Tumor Biol.* 37(1), 313–321 (2016).

95. Gallo A, Vella S, Miele M *et al.* Global profiling of viral and cellular non-coding RNAs in Epstein–Barr virus-induced lymphoblastoid cell lines and released exosome cargos. *Cancer Lett.* 388, 334–343 (2017).

96. Roth W, Huang M, Addae Konadu K, Powell M, Bond V. Micro RNA in exosomes from HIV-infected macrophages. *Int. J. Environ. Res. Public Health* 13(1), 32 (2016).

97. Fu Y, Zhang L, Zhang F *et al.* 293tExosome-mediated miR-146a transfer suppresses type I interferon response and facilitates EV71 infection. *PLoS Pathog.* 13(9), e1006611 (2017).

98. Han Z, Liu X, Chen X *et al.* miR-H28 and miR-H29 expressed late in productive infection are exported and restrict HSV-1 replication and spread in recipient cells. *Proc. Natl Acad. Sci. USA* 113(7), E894–E901 (2016).

99. Hoshina S, Sekizuka T, Kataoka M *et al.* Profile of exosomal and intracellular microRNA in gamma-herpesvirus-infected lymphoma cell lines. *PLoS ONE* 11(9), e0162574 (2016).

100. Chugh PE, Sin S-H, Ozgur S *et al.* Systemically circulating viral and tumor-derived microRNAs in KSHV-associated malignancies. *PLoS Pathog.* 9(7), e1003484 (2013).

101. Gourzones C, Gelin A, Bombik I *et al.* Extra-cellular release and blood diffusion of BART viral micro-RNAs produced by EBV-infected nasopharyngeal carcinoma cells. *Virol. J.* 7(1), 271 (2010).

•• **Shows that exosomal miRNAs play a role in infections with DNA viruses.**

102. Choi H, Lee H, Kim SR, Gho YS, Lee SK. Epstein-Barr virus-encoded microRNA BART15-3p promotes cell apoptosis partially by targeting BRUCE. *J. Virol.* 87(14), 8135–8144 (2013).

103. Choi H, Lee SK. TAX1BP1 downregulation by EBV-miR-BART15-3p enhances chemosensitivity of gastric cancer cells to 5-FU. *Arch. Virol.* 162(2), 369–377 (2017).

104. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA *et al.* Functional delivery of viral miRNAs via exosomes. *Proc. Natl Acad. Sci. USA* 107(14), 6328–6333 (2010).

105. Nuovo GJ, Garofalo M, Valeri N *et al.* Reovirus-associated reduction of microRNA-let-7d is related to the increased apoptotic death of cancer cells in clinical samples. *Mod. Pathol.* 25(10), 1333 (2012).

106. Volinia S, Galasso M, Sana ME *et al.* Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA. *Proc. Natl Acad. Sci. USA* 109(8), 3024–3029 (2012).

107. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. *Ann. Rev. Pathol.* 9, 287–314 (2014).

108. Harden ME, Munger K. Human papillomavirus 16 E6 and E7 oncoprotein expression alters microRNA expression in extracellular vesicles. *Virology* 508, 63–69 (2017).

109. Pakfetrat M, Yaghobi R, Salmanpoor Z, Roozbeh J, Torabinezhad S, Kadkhodaei S. Frequency of polyomavirus BK infection in kidney transplant patients suspected to nephropathy. *Int. J. Organ Transplant. Med.* 6(2), 77–84 (2015).

110. Hirsch HH, Brennan DC, Drachenberg CB *et al.* Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation* 79(10), 1277–1286 (2005).

111. Tian YC, Li YJ, Chen HC *et al.* Polyomavirus BK-encoded microRNA suppresses autoregulation of viral replication. *Biochem. Biophys. Res. Commun.* 447(3), 543–549 (2014).

112. Kim MH, Lee YH, Seo JW *et al.* Urinary exosomal viral microRNA as a marker of BK virus nephropathy in kidney transplant recipients. 12(12), e0190068 (2017).

113. Knowles WA, Pipkin P, Andrews N *et al.* Population-based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. *J. Med. Virol.* 71(1), 115–123 (2003).

114. Egli A, Infanti L, Dumoulin A *et al.* Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J. Infect. Dis.* 199(6), 837–846 (2009).

115. Polo C, Perez J, Mielnichuck A, Fedele C, Niubo J, Tenorio A. Prevalence and patterns of polyomavirus urinary excretion in immunocompetent adults and children. *Clin. Microbiol. Infect.* 10(7), 640–644 (2004).

116. Bayer A, Delorme-Axford E, Sleigher C *et al.* Human trophoblasts confer resistance to viruses implicated in perinatal infection. *Am. J. Obstet. Gynecol.* 212(1), 71.e71–71.e78 (2015).

117. Delorme-Axford E, Donker RB, Mouillet J-F *et al.* Human placental trophoblasts confer viral resistance to recipient cells. *Proc. Natl Acad. Sci. USA* 110(29), 12048–12053 (2013).
118. Payne L, Venugopal K. Neoplastic diseases: Marek's disease, avian leukosis and reticuloendotheliosis. *Rev. Sci. Tech.* 19(2), 544–560 (2000).
119. Powell P, Payne L, Frazier JA, Rennie M. T lymphoblastoid cell lines from Marek's disease lymphomas. *Nature* 251(5470), 79–80 (1974).
120. Neerukonda SN, Tavlarides-Hontz P, Mccarthy F, Pendarvis K, Parcells MS. Comparison of the transcriptomes and proteomes of serum exosomes from Marek's disease virus-vaccinated and protected and lymphoma-bearing chickens. *Genes* 10(2), 116 (2019).

•• **Shows that exosomal miRNAs play a role in infections with RNA viruses.**

121. Bernard MA, Zhao H, Yue SC, Anandaiah A, Koziel H, Tachado SD. Novel HIV-1 miRNAs stimulate TNFα release in human macrophages via TLR8 signaling pathway. *PLoS ONE* 9(9), e106006 (2014).
122. Hu G, Yao H, Chaudhuri A *et al.* Exosome-mediated shuttling of microRNA-29 regulates HIV Tat and morphine-mediated neuronal dysfunction. *Cell Death Dis.* 3(8), e381 (2012).
123. Chahar H, Bao X, Casola A. Exosomes and their role in the life cycle and pathogenesis of RNA viruses. *Viruses* 7(6), 3204–3225 (2015).
124. Shrivastava S, Steele R, Ray R, Ray RB. MicroRNAs: role in hepatitis C virus pathogenesis. *Genes Dis.* 2(1), 35–45 (2015).
125. Kim JH, Lee CH, Lee S-W. Hepatitis C virus infection stimulates transforming growth factor-β1 expression through up-regulating miR-192. *J. Microbiol.* 54(7), 520–526 (2016).
126. Xiong L, Zhen S, Yu Q, Gong Z. HCV-E2 inhibits hepatocellular carcinoma metastasis by stimulating mast cells to secrete exosomal shuttle microRNAs. *Oncol. Lett.* 14(2), 2141–2146 (2017).
127. Wang Y, Yi J, Chen X, Zhang Y, Xu M, Yang Z. The regulation of cancer cell migration by lung cancer cell-derived exosomes through TGF-β and IL-10. *Oncol. Lett.* 11(2), 1527–1530 (2016).
128. Cheung KL, Jarrett R, Subramaniam S *et al.* Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. *J. Exp. Med.* 213(11), 2399–2412 (2016).
129. Jia Z, Liu Y, Gao Q *et al.* miR-490-3p inhibits the growth and invasiveness in triple-negative breast cancer by repressing the expression of TNKS2. *Gene* 593(1), 41–47 (2016).
130. Xu X, Chen R, Li Z *et al.* MicroRNA-490-3p inhibits colorectal cancer metastasis by targeting TGFβR1. *BMC Cancer* 15(1), 1023 (2015).
131. Chen S, Chen X, Xiu Y-L, Sun K-X, Zhao Y. MicroRNA-490-3P targets CDK1 and inhibits ovarian epithelial carcinoma tumorigenesis and progression. *Cancer Lett.* 362(1), 122–130 (2015).
132. Zhang L-Y, Liu M, Li X, Tang H. miR-490-3p modulates cell growth and epithelial to mesenchymal transition of hepatocellular carcinoma cells by targeting endoplasmic reticulum-Golgi intermediate compartment protein 3 (ERGIC3). *J. Biol. Chem.* 288(6), 4035–4047 (2013).
133. Xu J, Zhang X, Wang H *et al.* HCRP1 downregulation promotes hepatocellular carcinoma cell migration and invasion through the induction of EGFR activation and epithelial-mesenchymal transition. *Biomed. Pharmacother.* 88, 421–429 (2017).
134. Cao C, Lu S, Sowa A *et al.* Priming with EGFR tyrosine kinase inhibitor and EGF sensitizes ovarian cancer cells to respond to chemotherapeutical drugs. *Cancer Lett.* 266(2), 249–262 (2008).
135. Lin F-C, Young HA. Interferons: success in anti-viral immunotherapy. *Cytokine Growth Factor Rev.* 25(4), 369–376 (2014).
136. Ho B-C, Yu I-S, Lu L-F *et al.* Inhibition of miR-146a prevents enterovirus-induced death by restoring the production of type I interferon. *Nat. Commun.* 5, 3344 (2014).
137. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* 9(6), 654 (2007).
138. Liu C-C, Guo M-S, Lin FH-Y *et al.* Purification and characterization of enterovirus 71 viral particles produced from vero cells grown in a serum-free microcarrier bioreactor system. *PLoS ONE* 6(5), e20005 (2011).
139. Mao L, Wu J, Shen L, Yang J, Chen J, Xu H. Enterovirus 71 transmission by exosomes establishes a productive infection in human neuroblastoma cells. *Virus Genes* 52(2), 189–194 (2016).
140. Rekker K, Saare M, Roost AM *et al.* Comparison of serum exosome isolation methods for microRNA profiling. *Clin. Biochem.* 47(1-2), 135–138 (2014).
141. György B, Módos K, Pállinger É *et al.* Detection and isolation of cell-derived microparticles are compromised by protein complexes resulting from shared biophysical parameters. *Blood* 117(4), e39–e48 (2011).
142. Narayanan A, Iordanskiy S, Das R *et al.* Exosomes derived from HIV-1-infected cells contain trans-activation response element RNA. *J. Biol. Chem.* 288(27), 20014–20033 (2013).

•• **Shows that exosomes play a role in HIV viral infection.**

143. Esser MT, Graham DR, Coren LV *et al.* Differential incorporation of CD45, CD80 (B7-1), CD86 (B7-2), and major histocompatibility complex class I and II molecules into human immunodeficiency virus type 1 virions and microvesicles: implications for viral pathogenesis and immune regulation. *J. Virol.* 75(13), 6173–6182 (2001).