Epigenetic Regulation of Chronic Pain
Review

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miRNA packaging into small extracellular vesicles and implications in pain

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Abstract

Extracellular vesicles (EVs) are a heterogenous group of lipid bilayer bound particles naturally released by cells. These vesicles are classified based on their biogenesis pathway and diameter. The overlap in size of exosomes generated from the exosomal pathway and macrovesicles that are pinched off from the surface of the plasma membrane makes it challenging to isolate pure populations. Hence, isolated vesicles that are less than 200 nm are called small extracellular vesicles (sEVs). Extracellular vesicles transport a variety of cargo molecules, and multiple mechanisms govern the packaging of cargo into sEVs. Here, we discuss the current understanding of how miRNAs are targeted into sEVs, including the role of RNA binding proteins and EXOmotif sequences present in miRNAs in sEV loading. Several studies in human pain disorders and rodent models of pain have reported alterations in sEV cargo, including miRNAs. The sorting mechanisms and target regulation of miR-939, a miRNA altered in individuals with complex regional pain syndrome, is discussed in the context of inflammation. We also provide a broad overview of the therapeutic strategies being pursued to utilize sEVs in the clinic and the work needed to further our understanding of EVs to successfully deploy sEVs as a pain therapeutic.

Keywords: Small extracellular vesicles, Exosomes, sEV biogenesis, miRNA, Pain, miR-939

1. Introduction

1.1. Extracellular vesicles and pain

Nearly every cell type within the human body releases extracellular vesicles (EVs). Extracellular vesicles are bilipid nanoparticles that range in diameter from nanometer to micrometer, with distinct biogenesis pathways and some common cargoes. Extracellular vesicles were first described as particles in blood plasma, where their removal via ultracentrifugation increased clotting time.[20] These "clotting factors" were subsequently identified as lipid vesicles. Extracellular vesicles were considered a type of molecular waste for most of the 20th century and as such little effort was dedicated to studying their functionality. We now know that EVs are a central form of information exchange between cells and can contain numerous molecules not limited to proteins, coding and noncoding nucleic acids, and lipids. These EVs serve several roles, including signaling, regulatory, and epigenetic functions, and have been implicated in many diseases.[26]

Defined as a complex sensory and emotional experience, pain serves as a protective warning mechanism to prevent potential harm to the body and promotes behaviors helping recovery.[119] Acute pain is usually time-limited and resolves as the underlying cause heals. Pain that persists beyond the normal healing time lasting weeks to years is considered chronic. Recent studies have shown that EVs released from different cell types including neurons, immune cells, and glial cells can modulate pain perception through various mechanisms.[16,172] In this review, we will discuss small extracellular vesicles (sEVs), their biogenesis, and miRNA cargo sorting under inflammation and pain reported in rodent models and human studies. Our objective is to provide a general overview of EV biogenesis and known cargo loading mechanisms in the context of miRNA, as well as our current understanding of how miRNAs are targeted and loaded into sEVs. In addition, we provide an overview of the therapeutic strategies being pursued to utilize sEVs in the clinic, and the work

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that needs to be done to further our understanding of how EVs could be used as a pain therapeutic.

1.2. Why we call it small extracellular vesicles instead of exosomes

The nomenclature surrounding EVs, historically, has not been well defined. Limited consensus existed on naming conventions for the multitude of particles released by cells, resulting in ambiguous characterization of self-reported particle types. As part of their initiative, the International Society for Extracellular Vesicles (ISEV) put forth standardized nomenclature for describing EVs, which has evolved from their original report (MISEV2014) to their most recent MISEV2023,[162] released February 2024. As such, we will refer to different EV categories with the terms defined by MISEV guidelines. It is important to note that EV classifications have distinct biogenesis mechanisms, origins, cargo, and functional purposes, which have been compiled and extensively described in these position papers. It is therefore imperative to use discretion when choosing the appropriate naming convention, since each classification has implications, especially regarding biogenesis.

Extracellular vesicles are generated by 2 broad mechanisms: outward budding of the plasma membrane or by an intracellular endocytic trafficking pathway involving fusion of multivesicular bodies (MVBs) with the plasma membrane. Extracellular release of intraluminal vesicles through MVBs generates exosomes ranging in size between 30 and 150 nm. Larger EVs such as microvesicles, microparticles, or ectosomes are pinched off from the plasma membrane with a size range of 100 to 1000 nm, whereas apoptotic bodies are 1 to 5 μm EVs released by cell death. Because of the overlap in diameter of exosomes and microvesicles, isolation methods almost always result in a heterogeneous population of EVs. Since it is difficult to distinguish the origin of vesicles under 200 nm to classify them as exosomes or microvesicles, the term sEVs is used to encompass all extracellular vesicles purified from various sources.[151]

1.3. Small extracellular vesicle biogenesis, packaging, and uptake

Exosomes are canonically formed through the endosomal pathway. Endosomes are intracellular, membrane-bound organelles generated from the invagination of the plasma membrane and are responsible for the trafficking of proteins to the lysosome. These early endosomes are generated through several endocytic pathways often classified as either clathrin-mediated or clathrin-independent endocytosis. Clathrin-mediated endocytosis is the primary endocytic pathway in mammalian cells, which involves the recruitment of clathrin and associated factors to the plasma membrane to promote membrane curving and scission.[98] Clathrin-independent endocytosis includes endocytosis mediated by caveolin and flotillin, among others.[98] Some of these pathways are constitutive, whereas others are triggered by specific signals. For example, interleukin-2 receptor (IL-2R) is one of the markers of clathrin-independent endocytosis. IL-2R is associated with cholesterol-rich lipid raft microdomains that promote optimal clustering of IL-2R at the cell surface, which is necessary for the efficient internalization of the receptor and thereby the regulation of IL-2–induced signaling.[84,130] Early endosomes then mature into late sorting endosomes through several maturation events.[64] These late sorting endosomes interact with the endoplasmic reticulum and the trans-Golgi network to sort cargo and fully mature.[12,167]

During late sorting endosome maturation, intraluminal vesicles (ILVs) begin to form by the inward budding of the limiting membrane of endosomes, creating a MVB (**Fig. 1A**). These MVBs have 2 fates: either fusion with the plasma membrane to release its ILVs as exosomes or fusion with a lysosome for degradation.[56] The production of ILVs is intimately involved with cargo loading, as intracellular components are taken up by vesicular invaginations,[8] and cargo is independently sorted into generating ILVs. The endosomal sorting complexes required for transport (ESCRT) are the canonical ILV cargo sorting machinery, comprised of 4 subcomplexes (ESCRT-0, -I, -II, and -III) and numerous accessory and scaffolding proteins.[12] Although the ESCRT complex is implicated in endosomal formation and many other cellular processes,[157] it is critical to EV biogenesis and is most prominently characterized in cargo sorting and ILV formation.[156]

Endosomal sorting complexes required for transport-mediated exosome biogenesis begins at the limiting membrane of the endosome, where ESCRT-0 congregates mono-ubiquitinated proteins through association with its subunit hepatocyte growth factor–regulated tyrosine kinase substrate (Hrs). These ubiquitin-tagged proteins are trafficked into forming ILVs with high phosphatidylinositol-3-phosphate content.[51,134] ESCRT-0 then recruits ESCRT-1 through its tumor susceptibility gene 101 (Tsg101) subunit. ESCRT-1 subsequently recruits ESCRT-II, both of which are responsible for invagination of the limiting membrane to form the ILV. ESCRT-III is finally recruited to the budding membrane, where it binds to ESCRT-II to facilitate the remainder of scission of the mature ILV. ESCRT-III is also responsible for the deubiquitination of sequestered cargo, a step required for proper delivery of proteins targeted to lysosomes for degradation.[54] ESCRT-III utilizes several accessory proteins, including components present in the cytosol and are incorporated into ILV membranes during formation. These complexes bind to and sort ubiquitinated proteins, which are targeted either to the ILV for secretion or to the lysosome for degradation.[56]

Importantly, the ESCRT pathway appears to contribute significantly to exosome production. A recent study performed an RNA interference screen targeting 23 components of the ESCRT machinery in exosome biogenesis, composition, and secretion.[23] They showed that depletion of vacuolar protein sorting–associated protein 4B (VPS4B) augmented secretion while not altering the features of exosomes. Silencing of genes for 2 components of ESCRT-0 (hepatocyte growth factor–regulated tyrosine kinase substrate or HRS), signal transducing adaptor molecule 1 (STAM1), or one component of ESCRT-I (TSG101) reduced exosome secretion.[23] In addition to subunits belonging to the 4 ESCRT complexes, the type II sphingomyelinase contributes to exosome biogenesis. Neutral sphingomyelinase 2 (nSMase2) is a key enzyme in the biogenesis and cargo loading of exosomes, hydrolyzing sphingomyelin into phosphocholine and ceramide, thereby initiating the formation of exosomes in multivesicular bodies.[152] Since there are multiple pathways regulating exosome biogenesis, it is technically challenging to completely block exosome release in vivo, thereby posing a challenge to conduct gain and loss of function studies to elucidate exosome function.

As mentioned above, the ESCRT machinery is also responsible for trafficking proteins for lysosomal degradation. Many subunits in the ESCRT machinery have ubiquitin binding motifs (notably, ESCRT-0, -I, and -II),[54] which help facilitate interactions with marked cargo. The role of ubiquitin in the sorting of cargo into ILVs destined for secretion is not fully understood.[51] Ubiquitination serves as a trafficking signal within MVBs and is essential to proper function of the endosomal system, as certain ESCRT components are regulated via ubiquitination.[59,78] Ubiquitinated proteins and

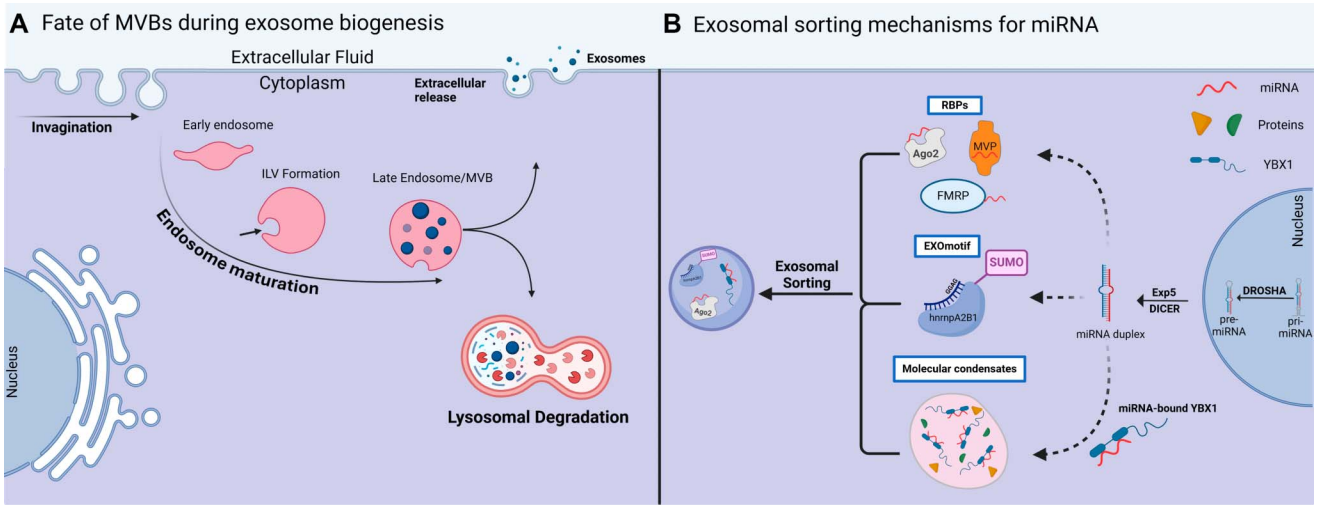

Figure 1. Multivesicular body maturation and miRNA packaging into exosomes. (A) Canonical exosome biogenesis begins with the invagination of the plasma membrane, forming a nascent endosome that often fuses with other newly formed invaginations. Recruitment of necessary proteins like ESCRT-0 and -I perform the prerequisite steps for ILV formation by promoting bending of the limiting membrane. ESCRTs-II, -III, and accessory proteins then sort ubiquitinated cargo, deubiquitinate them, and traffic the proteins into the vesicle. These complexes also assist in the scission of the limiting membrane, which completes the formation of the vesicle. The endosome continues to accumulate these vesicles and matures into a late sorting endosome, also called a multivesicular body (MVB). MVBs at this stage can either fuse with a lysosome (degradation) or traffic to the plasma membrane, where fusion of the limiting membrane leads to extracellular release of exosomes. (B) miRNA trafficking into ILVs has been reported to be mediated by several mechanisms: (1) after export from the nucleus to the cytosol, miRNAs can associate with a number of RBPs. These proteins then traffic to MVBs where they, along with the miRNA, are packaged into exosomes. The molecular mechanisms governing this trafficking are not well understood. (2) miRNAs sorted into exosomes are often enriched in small consensus sequences (EXOmotifs) that confer specificity for sorting into exosomes. RBPs such as sumoylated-hnrnpA2B1 are known to bind these EXOmotif-containing miRNAs and facilitate their localization into exosomes. (3) miRNAs can be enriched in molecular condensates, a type of phase-separated, membrane-less structure, which contains higher concentrations of certain molecules, such as proteins and RNA, compared to the cytosol. YBX1 binds to and localizes certain miRNAs to condensates, which increases their local concentration. YBX1-miRNA complexes are associated with processing (P-) bodies.

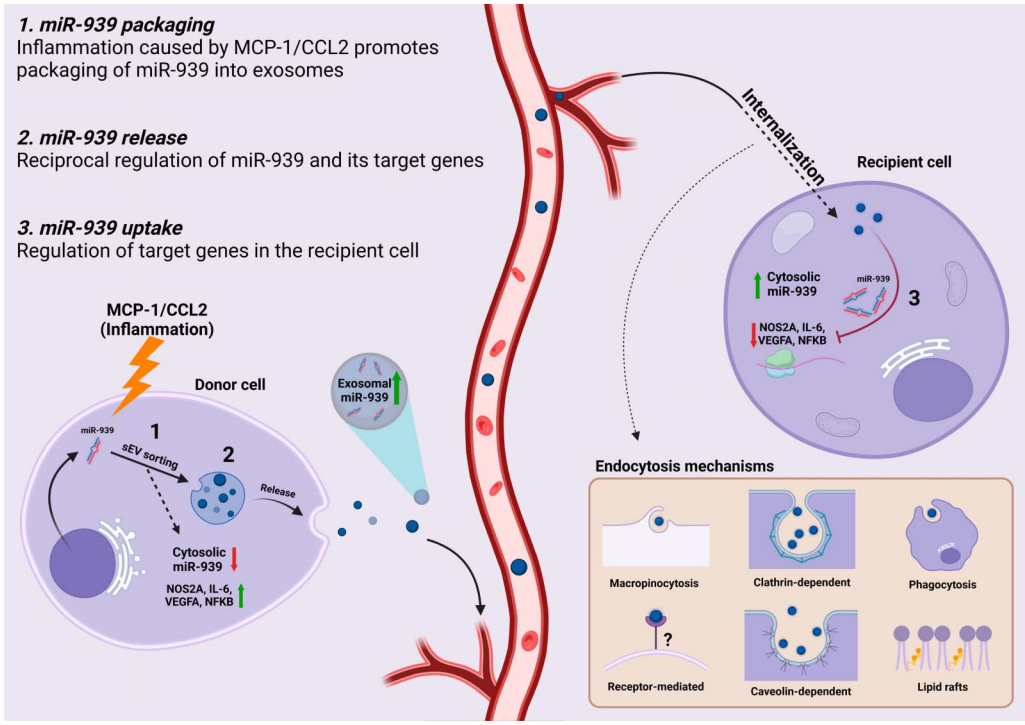

Figure 2. Schematic representations of miR-939 transport via exosomes and regulation of target genes in donor and recipient cells. (1) miR-939 packaging: selective inflammatory agents (MCP-1 but not LPS) upregulate miR-939 expression and thereby packaging and release of this miRNA in sEVs. (2) miR-939 release: since miR-939 can downregulate proinflammatory target mRNAs, packaging and release of miR-939 into circulation can make it conducive for the donor cell to upregulate proinflammatory genes in response to inflammatory stimuli. EV packaging could be a strategy used by the donor cell to regulate the balance between miRNA and its target genes and potentially signal cells at a distance by exporting miRNAs in sEVs. (3) miR-939 uptake: although it is unknown if miR-939 carrying sEVs are targeted to specific recipient cells, uptake of miR-939 enriched EVs can downregulate target genes in recipient cells at a distance, potentially reducing inflammation.

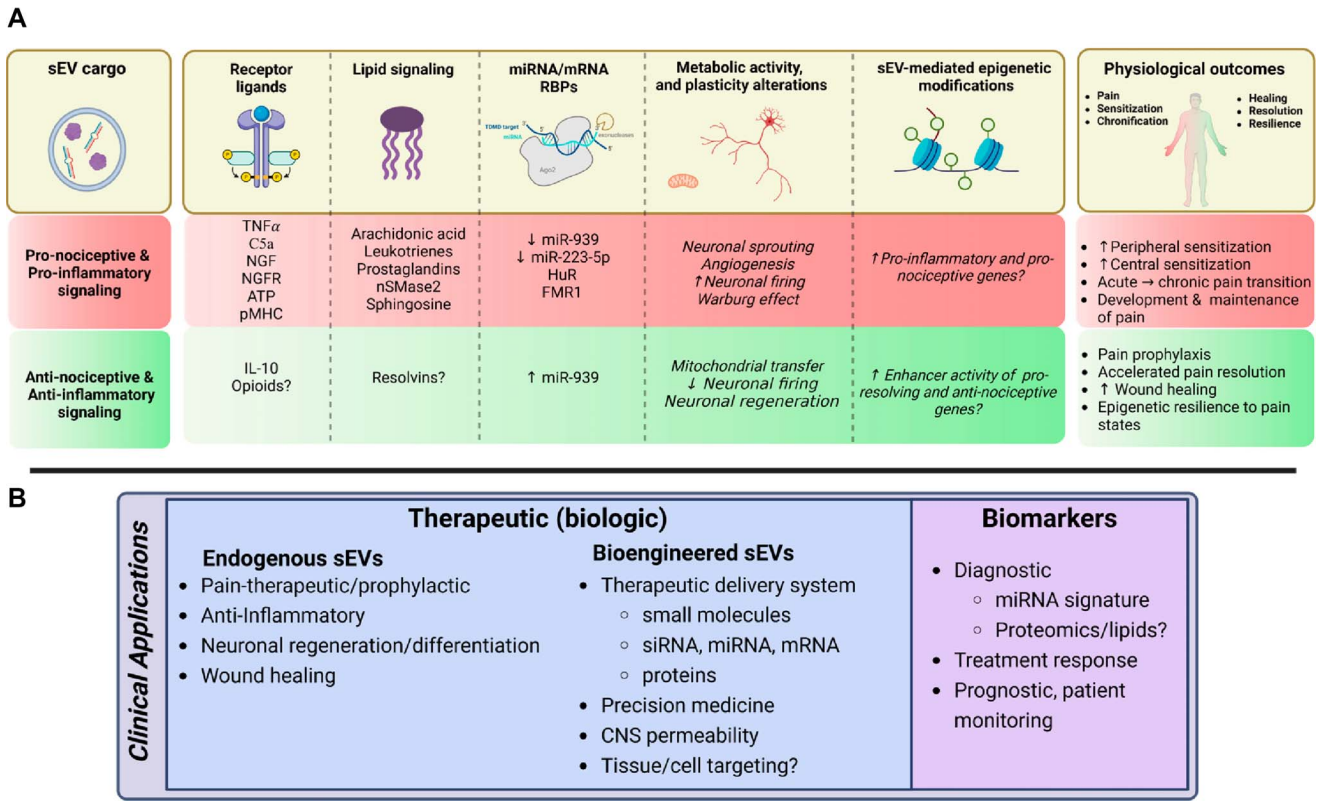

Figure 3. Proposed and hypothetical mechanisms of pain regulation by sEVs and potential clinical implications. (A) The effects of sEVs are governed by their intraluminal and membranous cargo, which is dependent on the donor cell type and its environment during sEV biogenesis. Many different classes of molecules identified in sEVs have been shown to regulate pain signaling. sEVs transport cytokines,[3] growth factors, and protein complexes such as peptide-loaded MHC molecules,[127] which may exacerbate inflammation and delay pain resolution, while certain cytokines like IL-10 are antinociceptive.[3] Endogenous bioactive lipids are present in sEVs and can promote inflammation and increase neuronal excitability contributing to pain.[124] Anti-inflammatory resolvins[136] could be present in sEVs and contribute to the restoration of cellular homeostasis after inflammation and injury. Several miRNAs, mRNA, and RBPs can have context specific proinflammatory or proresolving roles in recipient cells. Metabolic reprogramming induced by sEV uptake can result in a myriad of effects.[21,113] We hypothesize that these changes in signaling and metabolism culminate in epigenetic modifications, which, depending on the state of sEV cargo, could lead to upregulation of pro- or anti-inflammatory genes. (B) sEVs as pain therapeutic and biomarkers. sEVs produced from stem cells and immune cells such as macrophages have anti-inflammatory properties and are efficacious in rodent models of a wide variety of painful disorders.[173] In addition, the unique cargo profile of sEVs under disease could be utilized as biomarkers for pain disorders.[27,34,69,100,139,174] The ability of sEVs to cross the blood-brain barrier, combined with additional bioengineering of endogenous EVs, holds promise for a customizable therapeutic platform for treating pain.[16,172,173] Exogenous loading of several types of molecules have been proposed, including miRNAs, proteins, and small molecules, among others. ATP, Adenosine tri-phosphate; C5a, Complement component 5a; FMR1, Fragile X messenger ribonucleoprotein 1; HuR, Human antigen R; IL-10, Interleukin-10; NGF, Neuronal growth factor; NGFR, Neuronal growth factor receptor; nSMase2, Neutral sphingomyelinase 2; pMHC, Peptide major histocompatibility complex; TNFα, Tumor necrosis factor alpha.

molecules, especially when polyubiquitinated, are known to be targeted for degradation via the proteasome and lysosomes. Ubiquitin is normally removed from cargo before degradation,[118] yet EV cargoes are commonly ubiquitinated.[18,117] The exact role ubiquitination plays in EV cargo sorting is currently unclear. However, cargo loading is known to be nonrandom and heavily organized in mammalian cells, which suggests that such modifications are purposeful. Cargo from endocytosed exosomes are not necessarily functional, as they can be trafficked to lysosomes in recipient cells.[150] Therefore, it is possible that ubiquitination could confer a signal of degradation in recipient cells.

Once EVs are in circulation, they can exert their effect in both paracrine and autocrine manner,[4,44] after uptake by recipient cells. Extracellular vesicle uptake pathways can differ between cell types, with several known mechanisms including macropinocytosis, phagocytosis, clathrin-dependent endocytosis, clathrin-independent caveolin-mediated uptake, and lipid raft-mediated internalization.[40,106] For example, microglial cells have been observed to uptake EVs variably via macropinocytosis,[40] whereas neurons seem to prefer clathrin- and dynamin-mediated endocytosis.[42] Interestingly, exosomes have also been reported to "surf" on filopodia and enter human primary fibroblasts.[57] It has been reported that sEVs from different sources exhibit preferential interactions with specific recipient cells, although the mechanisms are unclear. Docking specificity of EVs to recipient cells is conferred by receptors and adhesion molecules present both on the EVs and recipient cells,[41] but there is no broad consensus in the field on all aspects underlying this phenomenon.[128] Membrane composition of recipient cells also plays a large role in efficient sEV internalization. A recent study using time-resolved atomic force microscopy showed a crucial role for local membrane fluidity with the strongest interaction and sEV fusion observed over the less fluid regions.[111] They reported that sEVs were capable of disrupting ordered domains in regions with high cholesterol concentration, indicating that the biophysical characteristics of recipient cell membranes can regulate sEVs uptake.[111]

1.4. Small extracellular vesicle cargo packaging—emphasis on miRNAs

Extracellular vesicles transport a variety of cargo. There are several purported mechanisms of EV cargo loading besides ESCRT-mediated mechanisms, and while the latter is far more

characterized, there is no consensus on which mechanisms predominate and when each alternative is used. The surge in interest in exosome biology, especially miRNAs as diagnostic biomarkers, followed the first report that miRNAs and mRNAs are present in sEVs in circulation and uptake of sEVs induces gene expression changes in recipient cells.[154] While there is an abundance of information on the extent of molecules that can be detected in sEVs and their effects, what mechanisms govern their sorting is still poorly understood.[86] Here, we will focus on miRNA cargo within sEVs, their sorting, and functional impact in recipient cells and discuss this in the context of pain biology.

2. Factors contributing to miRNA loading into small extracellular vesicles

2.1. Endosomal sorting complexes required for transport-dependent pathway and implications on miRNA loading

Several studies have shown that distinct components of ESCRT machinery promote the formation of exosomes, and that multiple pathways contribute to exosome biogenesis and release.[24,82] As mentioned above, the ESCRT complex is a major regulator of protein trafficking via the endosomal system through its ubiquitin interactions. However, it is less apparent if miRNA loading into exosomes is equally regulated by ESCRT. Because of its large role in EV biogenesis, it is reasonable to infer that the ESCRT complex may also exert some level of control over miRNA packaging. However, there is limited evidence to support a level of regulation similar to that of proteins sorted into endosomes. Some studies have indicated that certain ESCRT components help facilitate miRNA loading into sEVs, specifically in the presence of RNA binding proteins (RBPs).[61,65] ALIX, an ESCRT accessory protein, was reported to bind with RBP Argonaute2 in human liver stem-like cells, which is a protein involved in miRNA maturation and function.[65] In addition, the RBPs YBX1 and MEX3C were found to associate with ESCRT machinery,[61] and YBX1 has been directly implicated in sorting specific miRNAs into exosomes.[92,96,137] Fragile X mental retardation protein (FMRP) has been observed to bind Rab interacting lysosomal protein (RILP), which is involved in trafficking MVBs toward the cellular periphery.[151]

There are no studies that systematically determine the necessity of ESCRT for miRNA EV loading. From current available evidence, it is unclear how important ESCRT and its accessory proteins are to miRNA loading into exosomes. One report showed that knockdown of ALIX reduced the uptake of small RNA species via a stoplight CRISPR reporter system.[29] Gibbings et al. reported that localization of GW182, a component of the RNA-inducing silencing complex (RISC) complex, to MVBs is required for proper miRNA function and that knockdown of certain ESCRT proteins (ALIX and TSG101) inhibited miRNA activity.[49] This in itself does not provide evidence for miRNA loading into EVs via ESCRT, but it does demonstrate that these components have functional localization with miRNA relevant machinery. Taken together with the studies referenced above, there is support for the plausibility of ESCRT-mediated miRNA loading. A recent review covers interactions of miRNAs with ESCRT components and associated RNA binding proteins.[39]

2.2. Endosomal sorting complexes required for transport-independent pathway and implications on miRNA loading

Although central to sEV biogenesis, the ESCRT complex is not the only mechanism responsible for exosome production. Knockout of the entire ESCRT complex does not inhibit the formation of MVBs or sEVs in mammalian cells, and silencing ESCRT protein components Tsg101, Vps24, and Vps22 did not prevent MVB formation in mammalian cells.[143] Several ESCRT-independent pathways have been observed, but implications to miRNA loading are seldom. Neutral sphingomyelinase 2 (nSMase2) is responsible for the conversion of sphingomyelin into ceramide in endosomal membranes. Ceramide has been reported as essential to ILV formation of exosomes containing proteolipid protein (PLP)[80,83] and was the first ESCRT-independent pathway identified.[152] In addition, oligodendroglial cells with nSMase2 overexpression had increased presence of miRNAs in sEVs and conversely saw a decrease when nSmase2 was inhibited. Ceramide has been implicated with loading of angiogenic miRNAs in human breast cancer cells.[79] nSMase2 inhibition with GW4869 resulted in a decrease in exosomal markers and certain miRNAs in HEK293 cells and tumor cell lines,[36,62,80] which may support the role of ceramides in ILV formation and miRNA loading in certain cancers. Interestingly, human melanoma cells showed no requirement of nSMase2 for ILV production.[50] Rather, a CD63-dependent mechanism also exists for generating ILVs without ESCRT machinery, which could imply cell type–specific loading mechanisms. A number of other molecules are reported in mediating ESCRT-independent mechanisms, including phospholipase D2 (PLD2),[85] Rab31/flotillin,[161] small integral membrane protein of the lysosome/late endosome (SIMPLE),[177] and microtubule-associated proteins 1A/1B light chain 3B (LC3).[87] To the best of our knowledge, there are no reports on the relevance of these additional mechanisms to RNA presence in sEVs.

2.3. RNA binding proteins implicated in small extracellular vesicle cargo loading

One mechanism of RNA cargo loading into sEVs is mediated by RBPs (**Fig. 1B**). These are a functionally diverse group of proteins that can form complexes with both single and double stranded RNA. The ribonucleoprotein (RNP) complexes that are formed play a crucial role in regulating post-transcriptional gene expression. Numerous RBPs have been identified in recent years,[25,144] and advances in methodology have led to the identification of RBPs lacking canonical RNA binding domains.[55] Thus, in addition to structurally well-defined RNA binding domains, complex protein–RNA interactions mediated by intrinsically disordered regions, protein–protein interaction interfaces, and enzymatic cores can contribute to regulation of gene expression.[55] Several RBPs are implicated in the sorting of RNAs into EVs.[39] These include heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNPA2B1),[159] SYNCRIP,[58,132] HuR,[105] major vault protein (MVP),[142,149] and YBX1.[92,96,137] Selectivity for cargo sorting into EVs is best studied for RNA molecules including miRNAs.[126] Thirty RBPs detected in exosomes have been found to form complexes with both cellular RNA and exosomal-RNA species.[142] The authors concluded that since RBPs were found bound to their related RNAs, the RBP-RNA were loaded into exosomes as a complex. They suggest that RBPs could confer functionality within exosomes, including the maintenance of RNAs inside exosomes and shuttling RNAs into sEV recipient cells.[142] Post-translational modifications of RBPs such as SUMOylation, uridylation, and ubiquitination play a role in the selection of miRNA cargo for secretion in EVs.[77,159] There are a number of studies associating RBPs in trafficking miRNA to MVBs for cargo loading. hnRNPA2B1 is a well-known mediator of sorting specific miRNAs into sEVs. First shown by Villarroya-Beltri

et al. (2013), hnRNPA2B1 resides in the nucleus where it often binds to polyU pre-mRNA. This hnRNP has been proven to bind to miR-198, which was enhanced after the addition of small ubiquitin-like modifier (SUMO) protein to hnRNPA2B1. Other hnRNPA family proteins have been shown to have miRNA binding partners as well.[39] In addition, knockdown of hnRNPA2B1 in murine keratinocytes impaired their ability to package miRNAs.[176]

Major vault protein is another ubiquitously expressed RBP associated with transport of miRNAs into sEVs (**Fig. 1B**). Silencing of MVP reduced RNA shuttled into exosomes by approximately 50% while the total RNA in cells remained unchanged, and introduction of MVP increased shuttled RNA.[142] In another study, knockout of MVP resulted in the accumulation of miR-193a in the cytoplasm of donor cells, instead of being released in exosomes.[149] It is now well established that there are major differences between the RNA content of sEVs and their cells of origin. For example, exosomes lack 18S and 28S rRNA.[154] It is also known that different cell types may use RBPs with distinct binding preferences to secrete specific miRNA subsets in exosomes.[137]

We investigated mRNAs of all 30 identified RBPs in sEVs released by cultured primary neurons and astrocytes, with the majority showing no differential expression between the 2 cell types. We observed that mRNAs of 3 RBPs hnRNPH1, Argonaute 1 (AGO1), and Argonaute 4 (AGO4) were higher in neurons, while annexin A2 (ANXA2) and MVP were significantly lower compared to astrocytes. Similar to mRNA, MVP protein level was higher in astrocytes compared to neurons but there was no significant difference in ANXA2 protein between neurons and astrocytes. In addition, MVP was present in astrocytic sEVs but absent from neuronal sEVs, suggesting a role for MVP in differential RNA sorting into astrocytic vs neuronal sEVs. Thus, the mRNA and protein for MVP was upregulated in astrocytes compared to neurons, and MVP was present in astrocytic sEVs but not neuronal sEVs.[95] The role of MVP in shuttling RNA in sEVs released by neurons and astrocytes is not yet known.

2.4. RNA-inducing silencing complex complex and miRNAs

miRNAs associate with Argonaute 2 (AGO2) protein of the RISC to form AGO2-miRNA complexes. These complexes have been detected in both the extracellular space, such as plasma, as well as in secreted exosomes.[101] Thus, 2 distinct populations of circulating miRNAs are conferred stability by associating with AGO2 protein. This is an important consideration in biomarker discovery based on capture and analysis of circulating miRNAs.[11] The possibility of cells releasing functional miRNA-induced silencing complex into the circulation as extracellular AGO2-miRNA complexes cannot be ruled out.[11] Cell culture conditions can also affect the association of RBPs and RNAs with sEVs.

A large amount of nonvesicular RBPs were detected in conditioned media indicating that culture conditions greatly influence the RBP and RNA levels both inside and outside of sEVs.[71]

2.5. Role of EXOmotif in miRNA packaging

One reported sorting mechanism for miRNA loading into sEVs involves the presence of specific sequences in miRNA, called EXOmotifs. For example, 4 base-long motifs (GGAG) are present in EV-sorted miRNA at indiscriminate locations within the sequence (**Fig. 1B**).[159] EXOmotifs have a role for EV-targeted release through interactions with SUMOylated hnRNPA2B1. In addition to miRNA sorting EXOmotifs that determine miRNA secretion in sEVs, cellular retention sequences called CELLmotifs have been reported.[46] Different cell types employ preferential use of specific sorting sequences, thus defining the sEV miRNA profile of that cell type. Increase in miRNA levels mediated by EXOmotifs within sEVs enhanced inhibition of target genes in distant cells, suggesting that these miRNA code not only provides insights that link circulating sEV miRNAs to tissues of origin but can be used for improved targeting in RNA-mediated therapies.[46] Overall, multiple motifs have been reported to contribute to sEV sorting and cellular retention in a cell type–specific manner,[95] but not all miRNAs within sEVs have known motifs, indicating multiple mechanisms may be at play in vesicular encapsulation of miRNAs. A recent CRISPR genome-wide screening study used a library of artificially barcoded miRNAs containing EXOmotifs to quantitively assess the levels of the barcoded miRNAs in EVs and identify genes involved in the packaging and secretion of RNA.[93] In addition to validating several previously reported genes, they identified novel players including a critical role for Wnt signaling in the regulation of EV release.[93]

2.6. Molecular condensates

Another mechanism proposed for sEV cargo loading is via biomolecular condensates (**Fig. 1B**). These are non–membrane-bound organelles within cells formed by liquid–liquid phase separation, a process in which molecules such as proteins, RNA, and other biopolymers are concentrated into a liquid-like compartment. These include stress granules and processing bodies (P-bodies) among others and can exhibit a preference for, and exclusion of, specific proteins and RNAs.[5] Based on studies examining miR-223 sorting into exosomes by YBX1, it was proposed that local enrichment of cytosolic RBPs and their cognate RNAs via phase separation aids in their targeting and packaging by vesicles that bud into MVBs.[92] This can provide an efficient mechanism for entry of RBP bound RNAs into ILVs, which are then secreted as exosomes from cells.[92] The precise mechanisms by which cytosolic RBP-RNA complexes are recognized by MVBs and are loaded into exosomes remain largely unknown but is an active area of interest.

3. Small extracellular vesicles, miRNAs, and pain

A few recent publications have discussed the role of sEV miRNAs and their potential use as biomarkers and novel treatment strategies in various pain disorders and rodent models of pain.[27,172,175] In a comprehensive overview of human studies summarizing miRNA expression in the pathogenesis of chronic primary and secondary pain, the authors performed in silico analysis to identify target genes and pathways that are differentially regulated and could potentially contribute to the physiopathology of pain.[129] Molecular profiling of the epidermis using skin biopsy specimens from a homogeneous group of well-phenotyped individuals with gain-of-function variants in the voltage-gated sodium channel SCN9A gene identified alterations in 4 tissue-specific miRNAs. These miRNA strongly distinguished individuals with SCN9A gain-of-function mutations from healthy control donors. Epidermal miRNA–mRNA network analysis showed pain-related molecular signature and cross-talk between epidermal cells and axons in neuropathic pain.[9] A study investigating the correlation between the severity of symptoms in knee osteoarthritis (KOA) with changes in serum EV sub-populations identified changes in tetraspanin profiles between control and patient EVs. They did not investigate miRNA

composition but focused on EV proteins of the tetraspanin superfamily, which are among the most abundant membrane proteins in EV serum. The clear associations with KOA pain and functional limitations with EV tetraspanin profiles suggest that KOA influences the composition of EVs secreted into serum of individuals with KOA.[108]

Few studies in rodent models have investigated sEVs released by specific cells after culturing dissociated cells and isolating sEVs released into culture media. Small extracellular vesicles derived from both the central and peripheral tissues have been studied. miRNAs released from dorsal root ganglion (DRG) neurons in a rat chronic constriction injury model of neuropathic pain identified an increase in 6 miRNAs. Of these miRNAs, which were released from primary cultured DRG neurons prepared from rats 7 days after nerve injury, miR-221 was also upregulated in serum after nerve injury. miR-221 was not upregulated in an inflammatory pain model induced by the intraplantar injection of complete Freund adjuvant, suggesting specificity to models of nerve injury.[66] EV-mediated communication between neurons and glia have been reviewed recently.[1,114] The cargo characterization and function of sEVs released by satellite glial cells and Schwann cells, and their implications in pain pathogenesis and treatment, have also been reviewed recently.[48]

3.1. Small extracellular vesicles cargo alterations in the context of pain

Several human and rodent model studies have reported alterations in cargo composition in painful disorders. Studies on circulating miRNAs were performed using patient samples including blood, serum, plasma, and cerebrospinal fluid.[115] Pain disorders investigated include complex regional pain syndrome (CRPS),[33,120,125,164] migraine,[10,43,145] osteoarthritis,[76,108,122] rheumatoid arthritis,[148] inflammatory bowel diseases,[70] chemotherapy-induced pain,[17] bone cancer pain,[75] oral cancer,[14,37] head and neck cancer,[67] lumbar disc herniation,[104] and neuropathic pain.[69,139] These studies serve as a foundation for further investigations on how the cargo molecules facilitate intercellular communication under pain states. Further, the changes reported under pain state could be explored as potential biomarkers in combination with other clinical parameters.

While sEV transfer can modulate various aspects of pain processing, the cargo molecule most studied for their functional impact is miRNA transfer.[19,129] miRNAs can target genes involved in pain modulation, thereby influencing neuronal function and pain processing. Expression of inflammatory mediators regulated by pro- and anti-inflammatory miRNAs can contribute to the modulation of pain associated with inflammation.[174] Small extracellular vesicles released by neurons and glia have been reported to affect neuronal excitability and sensitization, thereby modulating signaling pathways in the nervous system.[1,48,135] Small extracellular vesicles released by immune cells taken up by neurons contribute to the neuroimmune crosstalk affecting pain responses, especially in conditions where immune activation is involved in pain states.[31,97] Stimulation of sensory neurons in vitro with capsaicin to activate neuronal TRPV1 receptors induced the release of exosomes containing miR-21-5p. Uptake of these exosomes by macrophages increased the expression of proinflammatory genes and proteins, suggesting neuron–macrophage communication in the DRG after nerve trauma.[138] Using neuron specific exosome reporter mice with extracellularly localized CD63-GFP + vesicles induced by neuronal CaMKII-Cre, it was demonstrated that $CD63^{+}$ vesicles can be secreted from neurons in the brain. Vesicles were primarily localized in soma and dendrites but not in axonal terminals in vitro and in vivo. miRNA cargos in secreted neuronal exosomes were transferred into astrocytes regulating astrocytic function.[102] A number of neuronal functions such as axon guidance, synapse formation, synapse elimination, neuronal firing, and potentiation have been reported to be affected by sEV uptake.[109] A recent study reported that macrophages transfer mitochondria to neurons through vesicles to resolve inflammatory pain.[155] Several recent studies showing therapeutic efficacy of sEVs in rodent models of pain are listed in **Table 1**.

It is now established that sEVs play significant roles in both physiological and pathological pain, acting as either beneficial or detrimental agents depending on the complex interactions and specific conditions under which sEVs exert their effects (**Fig. 3A**). The beneficial effects can be from anti-inflammatory properties dampening immune responses and promoting tissue repair to alleviate pain in conditions such as arthritis.[53,72] The cargo molecules in sEVs can influence pain signaling pathways by regulating gene expression and thereby modulating pain receptors, either directly or indirectly, potentially providing pain relief. The neuroprotective role of sEV cargo can facilitate the repair and regeneration of neural tissues.[173] On the contrary, sEVs released by injured or cancer cells can transport proinflammatory cytokines and other molecules that exacerbate pain. Tumor-derived exosomes have been shown to contribute to cancer-induced pain by promoting inflammation and sensitizing pain pathways.[14,37,47,75,90,113] Uptake of sEVs with molecules that exacerbate inflammation and pain can lead to the amplification or transition to chronic pain states. How sEVs travel long distances in circulation to impact pain is an area that awaits further exploration.

3.2. RNA binding proteins regulating pain

RNA binding proteins have been implicated in pain by regulating mRNA cap recognition, stabilization, stimulation, repression, and decay.[30] Two RBPs shown to regulate miRNA loading into sEVs have also been linked to pain modulation. Loss of function of RBP Fragile X mental retardation protein (FMRP) results in Fragile X syndrome. Studies using *Fmr1*-knockout mice have shown decreased responses to ongoing pain and a lag in the onset of injury-induced allodynia.[116] FMR1 was reported to regulate miRNA cargo loading into exosomes during inflammation (**Fig. 1B**). Sequence-specific interactions between miRNAs and FMR1 contributed to selective miRNA loading into exosomes via interaction with components of the ESCRT pathway under inflammation.[168] RNA binding protein HuR has been implicated in multiple sclerosis–related neuropathic pain and silencing spinal HuR alleviated hypernociceptive behavior in mice.[131] An interesting avenue to pursue would be to determine the function of RBPs transferred by sEVs into recipient cells and how RBP cargo modulates gene expression and function upon its transfer.

3.3. miR-939 packaging into small extracellular vesicles in complex regional pain syndrome and regulation of target genes in recipient cells

Complex regional pain syndrome is a chronic pain disorder with peripheral and central components. Individuals with CRPS have a range of symptoms including pain and inflammation[15] not alleviated by commonly used medications. A persistent pain state and chronic inflammation in individuals point to systemic aberrations. Investigation of circulating miRNA changes in whole blood from 41 individuals with CRPS compared to 20 control

Table 1

Recent studies showing therapeutic efficacy of small extracellular vesicles in rodent models of pain.

Year	Model system	sEV type	Proposed mechanisms	Effects	Ref.
2020	Nerve crush injury (rat)	SKP-SC EVs	Akt/mTOR/p70S6K pathway activation	Axonal regeneration	169
2021	CFA-induced inflammation (mouse)	RAW 264.7 sEVs	Anti-inflammatory miRNAs	Attenuated mechanical allodynia	68
2022	IDD via needle puncture (rat)	hMSC EVs	Notch signaling via Vasorin	Disc regeneration	91
2022	Carrageenan-induced inflammation (mouse)	BMDM sEVs	Transfer of mitochondria from $CD200R^+$ BMDMs to $iSec1^+$ sensory neurons	Resolution of pain	155
2022	Carrageenan-induced tendinopathy (rat)	iMSC sEVs	Inhibiting mast cell infiltration	Alleviated acute pain and inflammation	45
2022	SNI (mouse)	hPMSC sEVs	miR-26a-5p/Wnt5a signaling	Attenuated mechanical hypersensitivity	94
2022	IS (rat)	huMSC sEVs	NLRP3 inhibition	Neuroinflammation and mechanical allodynia alleviation	171
2023	Osteoarthritis (mouse)	hMSC EVs	Inhibition of neuronal hyperexcitability via NGF	Reduced pain behavior	2
2024	IDD via needle puncture (mouse)	PRP EVs	LncRNA MALAT1 regulating miR-217/SIRT1 to inhibit NF-κB/NLRP3-mediated pyroptosis	Promoted cell proliferation	147

BMDM, bone marrow–derived macrophages; CFA, complete Freund adjuvant; hMSC, human mesenchymal stem cells; hPMSC, human placental mesenchymal stem cells; huMSC, human umbilical cord mesenchymal stem cells; IDD, intervertebral disc degeneration; iMSC, induced mesenchymal stem cells; IS, interstitial cystitis; PRP-EVs, platelet-rich plasma-derived EVs; sEV, small extracellular vesicles; SKP-SC, skin precursor–derived Schwann cells; SNI, spared nerve injury.

donors identified differential expression of 18 miRNAs.[110] Of these miRNAs, miR-939 ranked first and showed a 4.3-fold downregulation in whole blood from individuals with CRPS.[110]

Investigations into the potential of miRNA to regulate target gene expression is particularly important to advance our understanding of their role in pain. Mechanistic studies focusing on individual miRNAs altered in circulation can provide insights on the underlying molecular mechanisms regulating pain and inflammation. Small noncoding miRNAs regulate gene expression by binding predominantly to the 3′ untranslated region (3′UTR) of mRNAs by a 6- to 8-basepair seed sequence complementarity to induce either mRNA degradation or translational repression. Therefore, miRNAs negatively regulate the expression of target genes.[13,165] Bioinformatic predictions showed that miR-939 can potentially target several genes encoding various proinflammatory mediators, including interleukin-6 (IL-6), vascular endothelial growth factor A (VEGFA), inducible nitric oxide synthase (NOS2A), and nuclear factor-κB2 (NFκB2). Among these putative targets, protein levels of IL-6 and VEGF were significantly upregulated in the whole blood of individuals with CRPS, and hence were negatively correlated with miR-939 levels.[110] Binding of miR-939 to the 3′ UTR of VEGFA, IL-6, NOS2A (iNOS), and NFκB2 was confirmed using a luciferase reporter assay.[99] To test the ability of miR-939 to modulate inflammatory mRNAs in vitro, we transfected THP-1 (human monocytic) and HUVEC (human umbilical vein endothelial) cells, representing 2 cell types in constant contact with circulating molecules. miRNAs can regulate gene expression by mRNA degradation or translational repression. Matched qPCR and ELISA/western data showed that miR-939-mediated gene regulation occured through both degradation and translational repression depending on the target gene. Overexpression of miR-939 cell-specifically reduced levels of both endogenous *IL-6* and *NOS2A* mRNA and their gene products in THP-1 and HUVEC cells, respectively. miR-939 overexpression reduced *VEGFA* levels in THP-1 cells, but not levels of its mRNA, suggesting regulation by translational repression.[99] Thus, both cellular context and modality of miRNA target gene regulation could have a significant role on downstream gene and protein expression.

There was significant upregulation of plasma levels of VEGFA and IL-6 proteins in all or a subset of individuals with CRPS compared with control,[110] in line with observations above showing miR-939 reduced the protein levels of IL-6 and VEGFA secreted into the media under inflammatory conditions.[99] The functional consequence of miR-939 downregulation on NOS2A was assessed by determining levels of the NOS substrate, L-arginine, in individuals with CRPS. There was a significant decrease in L-arginine levels in individuals with CRPS, suggesting that a reduction in miR-939 may contribute to an increase in NOS2A protein and NO and thereby result in inflammation. Thus, miR-939 may regulate multiple proinflammatory genes and that downregulation of miR-939 may increase expression of its target genes, including those involved in amplification of proinflammatory signaling cascades.[99]

As mentioned earlier, miRNAs in circulation can be transported via vesicles including exosomes, microvesicles, or apoptotic bodies. Alternately, miRNAs are rendered protection from RNases when present in association with high density lipoprotein (HDL) or the AGO protein complex. These could be nonspecific products resulting from cell death.[153] Thus, it is crucial to determine whether miRNAs are released randomly upon cell death or from a regulated compensatory mechanism in response to a stress signaling, such as inflammation. Determining the source of aberrant circulating miRNAs in chronic pain can have a significant clinical impact in identification of therapeutic targeting strategies and biomarker discovery. Live cells employ multiple distinct processes to release miRNA-containing

Table 2

Clinical trials evaluating the efficacy of small extracellular vesicles as a treatment for pain.

Study identifier	Disease/Condition	Treatment	Phase and enrollment (N)	Study status	Location
NCT05261360	Degenerative meniscal injury	Synovial fluid–derived mesenchymal stem cells–derived exosomes	Phase II, N = 30	Recruiting	Türkiye
NCT04849429	Intervertebral disc pathology	Platelet-rich plasma enriched with exosomes	Phase I, N = 30	Completed	India

exosomes. Though it is difficult to determine the exact source of exosomes in circulation, immune cells can contribute to circulating miRNAs in blood.[28]

To determine whether exosome miRNAs reflect the miRNA signature in whole blood of individuals with CRPS,[110] we profiled miRNAs in exosomes purified from the serum of 6 individuals with CRPS and 6 healthy controls.[100] As an endogenous control, we used the mean CT (cycle threshold) values of the 10 miRNAs with the lowest standard deviation. We observed differential expression of 127 miRNAs between CRPS and control-derived exosomes.[100] Sixteen of the 18 miRNAs dysregulated in individuals with CRPS from our previous study were detected in human serum–derived exosomes, but only 5, including miR-939, were significantly altered. In the exosome fraction of patient blood miR-939 was significantly upregulated; however, in whole blood miR-939 was downregulated.[110] Thus, although miR-939 was downregulated in whole blood (relative to total RNA including RNA derived from all circulating immune cells),[110] miR-939 was significantly upregulated in the serum-derived sEV fraction (normalized to sEV RNA).[100] This change in directionality should be considered in the context of total RNA normalization methods, as there are ~25-fold more miRNAs in whole blood compared to exosomal miRNA.[22]

Another study characterizing individuals with CRPS over a course of 2.5 years of standard treatment investigated exosomal miR-223 and miR-939 expression. This longitudinal study compared the changes in pain over this time period and then stratified individuals into "pain relief" (mean pain reduced by ≥ 2 numeric rating scale) or "persistence" (mean pain unchanged or worsened) groups. Although individuals reported lower pain and improved clinical characteristics after 2.5 years, they did not observe significant differences in miR-223 and miR-939 expression and in quantitative sensory testing. Stable exosomal miR-223 was observed in indviduals meeting the criteria for pain relief but decreased further when pain persisted.[125] Since a high incidence of CRPS after fracture has been reported, another study compared exosomes from individuals with CRPS to those with fracture. They found that miR-223-5p but not hsa-miR-144-5p was downregulated in individuals with CRPS compared to those with fracture.[33] It will be interesting to compare miRNA expression in individuals with CRPS from different studies to healthy control donors reported in literature if identical methods[38,141] are used in sEV and RNA isolation.

Higher levels of miR-939 in exosomes from individuals with CRPS suggests 2 possibilities: under inflammation (1) cells upregulate proinflammatory genes by selectively reducing cellular miR-939 through its secretion and (2) deliver the protective anti-inflammatory miR-939 to recipient cells using exosome-mediated signaling (**Fig. 2**). However, this raises the question as to why upregulation of exosomal miR-939 in individuals with CRPS does not reduce inflammation. This could be due to reduced/impaired efficacy of exosomal uptake by immune cells in individuals with CRPS. Inefficient delivery of anti-inflammatory miRNAs including miR-939 can increase proinflammatory gene expression in acceptor cells, leading to an outcome that differs from normal cellular response. It is possible that inflammation can upregulate the packaging of anti-inflammatory miRNAs into exosomes, and the uptake of these exosomes can decrease proinflammatory gene expression in recipient cells under normal conditions. Aberrant exosome uptake by specific immune cells could contribute to chronic inflammation in inividuals with CRPS.

Endogenous mRNAs were shown to modulate miRNA sorting into exosomes and transfer to acceptor cells.[140] Thus, miRNA secretion in sEVs could be a mechanism whereby cells rapidly dispose of excess miRNAs. This would prevent downregulation of target mRNAs and regulate cellular miRNA:mRNA homeostasis. If this mechanism is employed by different cell types, then perturbations in EV synthesis and release can affect cellular homeostasis and the proinflammatory target genes of miR-939. miR-939 packaging into sEVs could serve as a unique model to understand both miRNA alterations in an inflammatory state and how sEV-mediated gene regulation can influence pro- and anti-inflammatory homeostasis. Our studies suggest that there is a reciprocal regulation between miR-939 and its target mRNAs, and that blocking exosome release can upregulate cellular miR-939 and downregulate all 4 or a subset of target mRNAs.

Proinflammatory mediators like cytokines and chemokines play an important role in selectively recruiting immune cells such as monocytes and neutrophils.[32] Plasma levels of monocyte chemoattractant protein-1 (MCP-1), a chemokine also known as C-C motif chemokine ligand 2 (CCL2), is reported to be elevated in inividuals with CRPS.[110,112] Thus, we modeled an in vitro system by treating human monocytic THP-1 cells with MCP-1 to (1) assess if higher MCP-1 levels can influence miR-939 packaging into sEVs and (2) if the uptake of sEVs overexpressing miR-939 can alter gene expression in recipient cells. We observed that miR-939 was packaged into sEVs under inflammation (**Fig. 2**). This was specific for MCP-1 because LPS treatment of sEV donor cells did not induce miR-939 packaging into sEVs, indicating that sEV composition is governed by the stimulus.[121] Increase in miR-939 also caused the lowering of its target mRNA *IL-6*. sEV miR-939 was decreased upon LPS stimulation, which could be due to the elevated levels of cellular *IL-6* acting as a miRNA "sponge" and reducing available miR-939 that can be packaged.[121] Thus, sorting of miRNAs into exosomes can be modulated by changing either cellular miRNA expression or their respective endogenous target mRNA levels in the donor cells.[140] Collectively, these observations suggest that miRNA cargo sorting into sEVs is governed by cellular context and signaling.

As mentioned above, miRNAs contain sequence motifs called EXOmotifs, which control their localization in exosomes. These short sequence motifs are overrepresented in miRNAs that are commonly enriched in exosomes, allowing miRNAs to be specifically recognized (and bound) by transport protein hnRNPA2B1. These motifs control the loading of miRNAs into exosomes.[158] miR-939 sequence harbors one such EXOmotif (GGAG). Mutation of this EXOmotif under inflammatory stimulation reduced miR-939 expression in sEVs when compared to wild type miR-939, but did not completely abolish its incorporation into sEVs. This suggests that additional mechanisms could be at play in miR-939 packaging into sEVs.

nSMase2 aids exosomal secretion,[79] and nSMase2 inhibitors such as GW4869 and spiroepoxide[88] are widely used to inhibit the secretion of sEVs from cells. Additional commercially available inhibitors of nSMase2 include manumycin-A or brefeldin A.[103] Monensin is another pharmacological tool that can be employed. It is a Na^+/H^+ exchanger that induces changes in intracellular calcium (Ca^{2+}), resulting in the generation of large multivesicular bodies and enhanced exosome release.[133] Thus, pharmacological tools of exosome formation and release[6,63,88] can be used to elucidate if there is increase in cellular miRNA levels. When THP-1 cells were treated with GW4869, there was an increase in cellular miR-939 levels[121] indicating that inhibiting the secretion of sEVs resulted in the retention of miR-939 within the cells.

In conducting studies investigating uptake of sEVs expressing miR-939, we observed that proinflammatory gene expression

was always higher in recipient cells. This was due to the use of lipofectamine for miR-939 transfection into donor cells for sEV packaging, suggesting that other proinflammatory molecules may be packaged into sEVs in addition to miR-939.[121] Other studies have recommended caution in using transfection reagents for RNA interference studies, as these agents are known to cause subtle changes in inflammatory gene expression.[170] Thus, we pursued electroporation of naïve sEVs to efficiently package sEVs with miRNA with parameters optimized due to the smaller surface area of the vesicle compared to a eukaryotic cell surface.[160] Iodixanol was used in the electroporation buffer to reduce aggregation and maximize colloidal stability, since the low zeta potentials of sEV membranes (−30 mV) makes them susceptible to aggregation after electroporation.[60] Global RNA sequencing studies of THP-1 recipient cells after the uptake of miR-939–enriched sEVs showed a reversal of inflammation supporting a protective anti-inflammatory role for miR-939 (**Fig. 2**). This reversal in transcript expression, although closer to naïve cellular state, was not total under the experimental conditions employed.[121] This could be due to the fine-tuning function miRNAs exert on gene expression.

While in vitro studies can provide mechanistic insights on cargo sorting and the functional impact of specific miRNA enriched sEVs in cells of interest, it is crucial to determine the source of the miRNAs of interest that are altered in circulation under disease states. Although it is technically challenging to determine the source of sEVs in circulation because of the heterogeneous nature of both the sEVs present and the cells releasing them, controlled comparative studies can be performed by isolating immune cells of interest from study participants. We used peripheral blood mononuclear cells (PBMCs) to obtain $CD4^+$ T cells, $CD8^+$ T cells, monocytes, NK cells, and B cells and confirmed their purity by flow cytometry. The cells were immediately incubated in exosome-depleted RPMI individually for 24 hours, and the supernatant was used to isolate immune cell–specific sEVs for downstream analysis.[121]

The sEVs secreted by B cells, T cells, and NK cells showed elevated levels of miR-939 compared to monocyte-derived sEVs in controls. However, in individuals with CRPS, the ability of immune cells to secrete miR-939 within sEVs is diminished. Only B-cell–derived sEVs showed a significantly higher level of miR-939 among all the immune cell–derived sEVs. Studies using murine models of CRPS suggested that therapies directed at reducing B-cell activity may be beneficial in treating individuals with CRPS.[89] Thus, it is possible to obtain insights on the source of aberrant expression of specific miRNAs and potentially link them to cellular functions altered under disease states.

4. Clinical applications of small extracellular vesicles for pain

There is an emerging interest in understanding the role of sEVs in mediating pain and how they could be used as therapeutics and biomarkers (**Fig. 3B**). From a drug delivery perspective, sEVs can serve as natural nano-carriers for an array of therapeutic agents, including small molecules, proteins, and nucleic acids. The membrane protects EV cargo from degradation and could facilitate targeted delivery to specific tissues or cells. Small extracellular vesicles are considered to be safe, less immunogenic than their donor cells, and biodegradable when compared to lipid nanoparticles.[107] A major challenge in RNA therapeutics is the inability of these molecules to cross cell membranes.[35] An advantage of sEVs over lipid nanoparticles is that sEVs have an intrinsic ability to cross cellular and tissue barriers[7,52] and are endowed with endogenous cell-entry mechanisms. Small extracellular vesicles loaded with cannabinoids were successfully delivered to cancer cells, and a similar strategy has been proposed to alleviate cancer pain.[74] Although sEVs can cross the blood–brain barrier, challenges remain to ensure the drugs or therapeutic agent can reach the target of interest. For sEVs to be successful as a therapeutic strategy, several challenges need to be addressed; efficient scale-up of sEV production, controlled and reproducible loading of EVs with endogenous or exogenous drug, and poor pharmacokinetics (EVs have a short half-life of minutes in vivo after systemic administration)[107,146] are all impediments to be addressed. However, sEVs show much promise as an effective therapeutic. The lipids within EVs can exert a signaling role. Extracellular vesicles are reported to be a highly potent source of eicosanoids such as prostaglandins and leukotrienes, and are active both in vitro and in vivo.[123] Small extracellular vesicles derived from stem cells possess regenerative properties, and their immunomodulatory properties have been reported in various preclinical models including osteoarthritis and spinal cord injury.[81] Small extracellular vesicles derived from native or engineered immune cells (to express specific antigens) can modulate immune responses and hold promise as therapeutic agents for treating autoimmune diseases, inflammatory and pain disorders, and cancer.[68,73] Immune cell–derived sEVs can exert an immunoprotective or immunosuppressive effect on target cells and are capable of presenting peptide loaded major histocompatibility complexes (MHC) to T cells.[127,163]

5. Conclusions

Although sEV biogenesis has been well studied, the exact mechanisms that ultimately determine the fate of molecules for secretion in cells are not understood. Extracellular vesicles biogenesis is highly organized and regulated, and the general cargo loading mechanisms have been described. Yet it is unclear how, or why, certain molecules are loaded into sEVs. This is also true for sEV uptake by recipient cells; while there are several theories as to how sEVs could be targeted to certain recipients, a convincing paradigm that can explain such a mechanism and the factors involved is lacking. Obscurity of cargo loading mechanisms for the different types of macromolecules present in sEVs, including RNA, impedes researchers from utilizing sEVs to their full extent. These shortcomings are additionally confounded by the complicated nature of sEV uptake in recipient cells and the effects of different forms of internalization, which may affect the functionality of cargo. There are some significant gaps in knowledge that prevent full exploitation of these nanocarriers. An enhanced understanding of the biodistribution profile of exogenously administered sEVs and delaying rapid clearance can improve targeted sEV delivery.

A recent study evaluated a single injection of EVs from bone marrow–derived mesenchymal stem cells, an advanced investigational product, into the lumbar facet joint space as a treatment for chronic low back pain (**Table 2**).[166] A single injection did not induce any adverse effects during the 90-day evaluation period. Moreover, there was a significant and steady improvement of the scores across 3 different assessments of pain severity, interference, and disability over the entire 90-day study period, suggesting durable efficacy and minimal safety risks. A follow-up randomized controlled trial is needed to further evaluate the safety and efficacy of EVs.[166] To widen the therapeutic potential of EVs, it will be necessary to determine the feasibility of engineering cell type–specific targeting which is seldom observed

at this point in time. If such mechanisms exist, elucidating them would be incredibly beneficial to broadening the application of EVs as a treatment option for more diseases and routine clinical applications.

Disclosures

The authors have no conflict of interest to declare.

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References

[1] Ahmad S, Srivastava RK, Singh P, Naik UP, Srivastava AK. Role of extracellular vesicles in glia-neuron intercellular communication. Front Mol Neurosci. 2022;15:1–11.
[2] Ai M, Hotham WE, Pattison LA, Ma Q, Henson FMD, Smith ESJ. Role of human mesenchymal stem cells and derived extracellular vesicles in reducing sensory neuron hyperexcitability and pain behaviors in murine osteoarthritis. Arthritis Rheumatol 2023;75:352–63.
[3] Aiello A, Giannessi F, Percario ZA, Affabris E. An emerging interplay between extracellular vesicles and cytokines. Cytokine Growth Factor Rev 2020;51:49–60.
[4] Al-Nedawi K, Meehan B, Kerbel RS, Allison AC, Rak J. Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proc Natl Acad Sci USA 2009;106:3794–9.
[5] Alberti S, Gladfelter A, Mittag T. Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. Cell 2019;176:419–34.
[6] Alexander M, Hu R, Runtsch MC, Kagele DA, Mosbruger TL, Tolmachova T, Seabra MC, Round JL, Ward DM, O'Connell RM. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Nat Commun 2015;6:7321.
[7] Alvarez-Erviti L, Seow Y, Yin HF, Betts C, Lakhal S, Wood MJA. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 2011;29:341–5.
[8] Anand S, Samuel M, Kumar S, Mathivanan S. Ticket to a bubble ride: cargo sorting into exosomes and extracellular vesicles. Biochim Biophys Acta (BBA) Proteins Proteomics. 2019;1867:1–10.
[9] Andelic M, Salvi E, Marcuzzo S, Marchi M, Lombardi R, Cartelli D, Cazzato D, Mehmeti E, Gelemanovic A, Paolini M, Pardo C, D'Amato I, Hoeijmakers JGJ, Dib-Hajj S, Waxman SG, Faber CG, Lauria G. Integrative miRNA–mRNA profiling of human epidermis: unique signature of SCN9A painful neuropathy. Brain 2023;146:3049–62.
[10] Andersen H, Duroux M, Gazerani P. Serum MicroRNA signatures in migraineurs during attacks and in pain-free periods. Mol Neurobiol 2015;53:1494–500.
[11] Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA 2011;108:5003–8.
[12] Arya SB, Collie SP, Parent CA. The ins-and-outs of exosome biogenesis, secretion, and internalization. Trends Cell Biol 2024;34:90–108.
[13] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215–33.
[14] Bhattacharya A, Janal MN, Veeramachaneni R, Dolgalev I, Dubeykovskaya Z, Tu NH, Kim H, Zhang S, Wu AK, Hagiwara M, Kerr AR, DeLacure MD, Schmidt BL, Albertson DG. Oncogenes overexpressed in metastatic oral cancers from patients with pain: potential pain mediators released in exosomes. Scientific Rep. 2020;10:1–13.
[15] Birklein F, Schlereth T. Complex regional pain syndrome-significant progress in understanding. PAIN 2015;156(suppl 1):S94–103.
[16] Bryk M, Karnas E, Mlost J, Zuba-Surma E, Starowicz K. Mesenchymal stem cells and extracellular vesicles for the treatment of pain: current status and perspectives. Br J Pharmacol 2022;179:4281–99.
[17] Buchheit T, Huh Y, Breglio A, Bang S, Xu J, Matsuoka Y, Guo R, Bortsov A, Reinecke J, Wehling P, Jun Huang T, Ji RR. Intrathecal administration of conditioned serum from different species resolves chemotherapy-induced neuropathic pain in mice via secretory exosomes. Brain Behav Immun 2023;111:298–311.
[18] Buschow SI, Liefhebber JM, Wubbolts R, Stoorvogel W. Exosomes contain ubiquitinated proteins. Blood Cells Mol Dis 2005;35:398–403.
[19] Cata JP, Gorur A, Yuan X, Berg NK, Sood AK, Eltzschig HK. Role of micro-RNA for pain after surgery: narrative review of animal and human studies. Anesth Analg 2020;130:1638–52.
[20] Chargaff E, West R. The biological significance of the thromboplastic protein of blood. J Biol Chem 1946;166:189–97.
[21] Chen X, Luo Y, Zhu Q, Zhang J, Huang H, Kan Y, Li D, Xu M, Liu S, Li J, Pan J, Zhang L, Guo Y, Wang B, Qi G, Zhou Z, Zhang C-Y, Fang L, Wang Y, Chen X. Small extracellular vesicles from young plasma reverse age-related functional declines by improving mitochondrial energy metabolism. Nat Aging 2024;4:814–38.
[22] Cheng L, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. J Extracellular Vesicles. 2014;3:23743.
[23] Colombo M, Moita C, van Niel G, Kowal J, Vigneron J, Benaroch P, Manel N, Moita LF, Théry C, Raposo G. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J Cell Sci 2013;126:5553–65.
[24] Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev cell Dev Biol 2014;30:255–89.
[25] Cook KB, Kazan H, Zuberi K, Morris Q, Hughes TR. RBPDB: a database of RNA-binding specificities. Nucleic Acids Res 2011;39:D301–8.
[26] Couch Y, Buzàs EI, Di Vizio D, Gho YS, Harrison P, Hill AF, Lötvall J, Raposo G, Stahl PD, Théry C, Witwer KW, Carter DRF. A brief history of nearly EV-erything—the rise and rise of extracellular vesicles. J Extracellular vesicles. 2021;10:1–12.
[27] D'Agnelli S, Gerra MC, Bignami E, Arendt-Nielsen L. Exosomes as a new pain biomarker opportunity. Mol Pain. 2020;16:1–9.
[28] de Candia P, Torri A, Pagani M, Abrignani S. Serum microRNAs as biomarkers of human lymphocyte activation in health and disease. Front Immunol. 2014;5:1–6.
[29] de Jong OG, Murphy DE, Mäger I, Willms E, Garcia-Guerra A, Gitz-Francois JJ, Lefferts J, Gupta D, Steenbeek SC, van Rheenen J, El Andaloussi S, Schiffelers RM, Wood MJA, Vader P. A CRISPR-Cas9-based reporter system for single-cell detection of extracellular vesicle-mediated functional transfer of RNA. Nat Commun 2020;11:1113.
[30] de la Peña JB, Campbell ZT. RNA-binding proteins as targets for pain therapeutics. Neurobiol Pain 2018;4:2–7.
[31] Delpech JC, Herron S, Botros MB, Ikezu T. Neuroimmune crosstalk through extracellular vesicles in health and disease. Trends Neurosci 2019;42:361–72.
[32] Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009;29:313–26.
[33] Dietz C, Müller M, Reinhold A-K, Karch L, Schwab B, Forer L, Vlckova E, Brede E-M, Jakubietz R, Üçeyler N, Meffert R, Bednarik J, Kress M, Sommer C, Dimova V, Birklein F, Rittner HL. What is normal trauma healing and what is complex regional pain syndrome I? An analysis of clinical and experimental biomarkers. PAIN 2019;160:2278–89.
[34] Douglas SR, Shenoda BB, Qureshi RA, Sacan A, Alexander GM, Perreault M, Barrett JE, Aradillas-Lopez E, Schwartzman RJ, Ajit SK. Analgesic response to intravenous ketamine is linked to a circulating microRNA signature in female patients with complex regional pain syndrome. J Pain 2015;16:814–24.
[35] Dowdy SF. Overcoming cellular barriers for RNA therapeutics. Nat Biotechnol 2017;35:222–9.
[36] Dreux M, Garaigorta U, Boyd B, Décembre 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;12:558–70.
[37] Dubeykovskaya ZA, Tu NH, Garcia PDR, Schmidt BL, Albertson DG. Oral cancer cells release vesicles that cause pain. Adv Biol. 2022;6:1–12.

[38] Eldh M, Lötvall J, Malmhäll C, Ekström K. Importance of RNA isolation methods for analysis of exosomal RNA: evaluation of different methods. Mol Immunol 2012;50:278–86.
[39] Fabbiano F, Corsi J, Gurrieri E, Trevisan C, Notarangelo M, D'Agostino VG. RNA packaging into extracellular vesicles: an orchestra of RNA-binding proteins? J Extracell Vesicles. 2020;10:1–29.
[40] Fitzner D, Schnaars M, van Rossum D, Krishnamoorthy G, Dibaj P, Bakhti M, Regen T, Hanisch U-K, Simons M. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. J Cell Sci 2011;124:447–58.
[41] French KC, Antonyak MA, Cerione RA. Extracellular vesicle docking at the cellular port: extracellular vesicle binding and uptake. Semin Cell Dev Biol 2017;67:48–55.
[42] Frühbeis C, Fröhlich D, Kuo WP, Amphornrat J, Thilemann S, Saab AS, Kirchhoff F, Möbius W, Goebbels S, Nave K-A, Schneider A, Simons M, Klugmann M, Trotter J, Krämer-Albers E-M. Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte–neuron communication. PLoS Biol. 2013;11:1–19.
[43] Gallardo VJ, Gómez-Galván JB, Asskour L, Torres-Ferrús M, Alpuente A, Caronna E, Pozo-Rosich P. A study of differential microRNA expression profile in migraine: the microMIG exploratory study. J Headache Pain 2023;24:11.
[44] Gangoda L, Boukouris S, Liem M, Kalra H, Mathivanan S. Extracellular vesicles including exosomes are mediators of signal transduction: are they protective or pathogenic? Proteomics 2015;15:260–71.
[45] Gao R, Ye T, Zhu Z, Li Q, Zhang J, Yuan J, Zhao B, Xie Z, Wang Y. Small extracellular vesicles from iPSC-derived mesenchymal stem cells ameliorate tendinopathy pain by inhibiting mast cell activation. Nanomedicine (Lond) 2022;17:513–29.
[46] Garcia-Martin R, Wang G, Brandão BB, Zanotto TM, Shah S, Kumar Patel S, Schilling B, Kahn CR. MicroRNA sequence codes for small extracellular vesicle release and cellular retention. Nature 2022;601:446–51.
[47] García-Silva S, Benito-Martín A, Nogués L, Hernández-Barranco A, Mazariegos MS, Santos V, Hergueta-Redondo M, Ximénez-Embún P, Kataru RP, Lopez AA, Merino C, Sánchez-Redondo S, Graña-Castro O, Matei I, Nicolás-Avila JÁ, Torres-Ruiz R, Rodríguez-Perales S, Martínez L, Pérez-Martínez M, Mata G, Szumera-Ciećkiewicz A, Kalinowska I, Saltari A, Martínez-Gómez JM, Hogan SA, Saragovi HU, Ortega S, Garcia-Martin C, Boskovic J, Levesque MP, Rutkowski P, Hidalgo A, Muñoz J, Megías D, Mehrara BJ, Lyden D, Peinado H. Melanoma-derived small extracellular vesicles induce lymphangiogenesis and metastasis through an NGFR-dependent mechanism. Nat Cancer 2021;2:1387–405.
[48] Gazerani P. Contribution of small extracellular vesicles from Schwann cells and satellite glial cells to pain processing. Neuroglia 2024;5:1–12.
[49] Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat Cell Biol 2009;11:1143–9.
[50] Guillaume vN, Charrin S, Simoes S, Romao M, Rochin L, Saftig P, Marks MS, Rubinstein E, Raposo G. The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis. Develop Cell 2011;21:708–21.
[51] Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. Cell Commun Signal 2021;19:47.
[52] Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B 2016;6:287–96.
[53] He L, He T, Xing J, Zhou Q, Fan L, Liu C, Chen Y, Wu D, Tian Z, Liu B, Rong L. Bone marrow mesenchymal stem cell-derived exosomes protect cartilage damage and relieve knee osteoarthritis pain in a rat model of osteoarthritis. Stem Cell Res Ther 2020;11:276.
[54] Henne WM, Buchkovich NJ, Emr SD. The ESCRT pathway. Develop Cell 2011;21:77–91.
[55] Hentze MW, Castello A, Schwarzl T, Preiss T. A brave new world of RNA-binding proteins. Nat Rev Mol Cell Biol 2018;19:327–41.
[56] Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cell Mol Life Sci 2018;75:193–208.
[57] Heusermann W, Hean J, Trojer D, Steib E, von Bueren S, Graff-Meyer A, Genoud C, Martin K, Pizzato N, Voshol J, Morrissey DV, Andaloussi SE, Wood MJ, Meisner-Kober NC. Exosomes surf on filopodia to enter cells at endocytic hot spots, traffic within endosomes, and are targeted to the ER. J Cell Biol 2016;213:173–84.
[58] Hobor F, Dallmann A, Ball NJ, Cicchini C, Battistelli C, Ogrodowicz RW, Christodoulou E, Martin SR, Castello A, Tripodi M, Taylor IA, Ramos A. A cryptic RNA-binding domain mediates Syncrip recognition and exosomal partitioning of miRNA targets. Nat Commun 2018;9:831.
[59] Hoeller D, Crosetto N, Blagoev B, Raiborg C, Tikkanen R, Wagner S, Kowanetz K, Breitling R, Mann M, Stenmark H, Dikic I. Regulation of ubiquitin-binding proteins by monoubiquitination. Nat Cell Biol 2006;8:163–9.
[60] Hood JL, Scott MJ, Wickline SA. Maximizing exosome colloidal stability following electroporation. Anal Biochem 2014;448:41–9.
[61] Hornung T, O'Neill HA, Logie SC, Fowler KM, Duncan JE, Rosenow M, Bondre AS, Tinder T, Maher V, Zarkovic J, Zhong Z, Richards MN, Wei X, Miglarese MR, Mayer G, Famulok M, Spetzler D. ADAPT identifies an ESCRT complex composition that discriminates VCaP from LNCaP prostate cancer cell exosomes. Nucleic Acids Res 2020;48:4013–27.
[62] Hoshino D, Kirkbride KC, Costello K, Clark ES, Sinha S, Grega-Larson N, Tyska MJ, Weaver AM. Exosome secretion is enhanced by invadopodia and drives invasive behavior. Cell Rep 2013;5:1159–68.
[63] Hu G, Yao H, Chaudhuri AD, Duan M, Yelamanchili SV, Wen H, Cheney PD, Fox HS, Buch S. Exosome-mediated shuttling of microRNA-29 regulates HIV Tat and morphine-mediated Neuronal dysfunction. Cell Death Dis 2012;3:e381.
[64] Huotari J, Helenius A. Endosome maturation. EMBO J 2011;30:3481–500.
[65] Iavello A, Frech VSL, Gai C, Deregibus MC, Quesenberry PJ, Camussi G. Role of Alix in miRNA packaging during extracellular vesicle biogenesis. Int J Mol Med 2016;37:958–66.
[66] Ikuma Y, Sakai A, Sakamoto A, Suzuki H. Increased extracellular release of microRNAs from dorsal root ganglion cells in a rat model of neuropathic pain caused by peripheral nerve injury. PLoS One. 2023;18:1–10.
[67] Inyang KE, Evans CM, Heussner M, Petroff M, Reimers M, Vermeer PD, Tykocki N, Folger JK, Laumet G. HPV+ head and neck cancer-derived small extracellular vesicles communicate with TRPV1+ neurons to mediate cancer pain. PAIN 2024;165:608–20.
[68] Jean-Toussaint R, Lin Z, Tian Y, Gupta R, Pande R, Luo X, Hu H, Sacan A, Ajit SK. Therapeutic and prophylactic effects of macrophage-derived small extracellular vesicles in the attenuation of inflammatory pain. Brain Behav Immun 2021;94:210–24.
[69] Jean-Toussaint R, Tian Y, Chaudhuri AD, Haughey NJ, Sacan A, Ajit SK. Proteome characterization of small extracellular vesicles from spared nerve injury model of neuropathic pain. J Proteomics. 2020;211:1–9.
[70] Jiang L, Shen Y, Guo D, Yang D, Liu J, Fei X, Yang Y, Zhang B, Lin Z, Yang F, Wang X, Wang K, Wang J, Cai Z. EpCAM-dependent extracellular vesicles from intestinal epithelial cells maintain intestinal tract immune balance. Nat Commun. 2016;7:1–16.
[71] Jimenez L, Barman B, Jung YJ, Cocozza L, Krystofiak E, Saffold C, Vickers KC, Wilson JT, Dawson TR, Weaver AM. Culture conditions greatly impact the levels of vesicular and extravesicular Ago2 and RNA in extracellular vesicle preparations. J Extracell Vesicles. 2023;12:1–17.
[72] Jin Z, Ren J, Qi S. Exosomal miR-9-5p secreted by bone marrow–derived mesenchymal stem cells alleviates osteoarthritis by inhibiting syndecan-1. Cell Tissue Res 2020;381:99–114.
[73] Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367:1–15.
[74] Kaur S, Nathani A, Singh M. Exosomal delivery of cannabinoids against cancer. Cancer Lett. 2023;566:1–17.
[75] Khasabova IA, Khasabov SG, Johns M, Juliette J, Zheng A, Morgan H, Flippen A, Allen K, Golovko MY, Golovko SA, Zhang W, Marti J, Cain D, Seybold VS, Simone DA. Exosome-associated lysophosphatidic acid signaling contributes to cancer pain. PAIN 2023;164:2684–95.
[76] Kolhe R, Owens V, Sharma A, Lee TJ, Zhi W, Ghilzai U, Mondal AK, Liu Y, Isales CM, Hamrick MW, Hunter M, Fulzele S. Sex-specific differences in extracellular vesicle protein cargo in synovial fluid of patients with osteoarthritis. Life (Basel). 2020;10:1–13.
[77] Koppers-Lalic D, Hackenberg M, Bijnsdorp IreneV, van Eijndhoven MAJ, Sadek P, Sie D, Zini N, Middeldorp JM, Ylstra B, de Menezes RX, Würdinger T, Meijer GA, Pegtel DM. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. Cell Rep 2014;8:1649–58.
[78] Korbei B. Ubiquitination of the ubiquitin-binding machinery: how early ESCRT components are controlled. Essays Biochem 2022;66:169–77.
[79] Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic MicroRNAs regulate cancer cell metastasis. J Biol Chem 2013;288:10849–59.
[80] 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 2010;285:17442–52.
[81] Kou M, Huang L, Yang J, Chiang Z, Chen S, Liu J, Guo L, Zhang X, Zhou X, Xu X, Yan X, Wang Y, Zhang J, Xu A, Tse H-f, Lian Q. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and

regeneration: a next generation therapeutic tool? Cell Death Dis 2022; 13:580.
[82] Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, Dingli F, Loew D, Tkach M, Théry C. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci USA 2016;113: E968–77.
[83] Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol 2014;29:116–25.
[84] Lamaze C, Dujeancourt A, Baba T, Lo CG, Benmerah A, Dautry-Varsat A. Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. Mol cell 2001;7: 661–71.
[85] Laulagnier K, Grand D, Dujardin A, Hamdi S, Vincent-Schneider H, Lankar D, Salles J-P, Bonnerot C, Perret B, Record M. PLD2 is enriched on exosomes and its activity is correlated to the release of exosomes. FEBS Lett 2004;572:11–4.
[86] Leidal AM, Debnath J. Unraveling the mechanisms that specify molecules for secretion in extracellular vesicles. Methods 2020;177: 15–26.
[87] Leidal AM, Huang HH, Marsh T, Solvik T, Zhang D, Ye J, Kai F, Goldsmith J, Liu JY, Huang Y-H, Monkkonen T, Vlahakis A, Huang EJ, Goodarzi H, Yu L, Wiita AP, Debnath J. The LC3-conjugation machinery specifies the loading of RNA-binding proteins into extracellular vesicles. Nat Cell Biol 2020;22:187–99.
[88] Li J, Liu K, Liu Y, Xu Y, Zhang F, Yang H, Liu J, Pan T, Chen J, Wu M, Zhou X, Yuan Z. Exosomes mediate the cell-to-cell transmission of IFN-α-induced antiviral activity. Nat Immunol 2013;14:793–803.
[89] Li WW, Guo TZ, Shi X, Czirr E, Stan T, Sahbaie P, Wyss-Coray T, Kingery WS, Clark JD. Autoimmunity contributes to nociceptive sensitization in a mouse model of complex regional pain syndrome. PAIN 2014;155: 2377–89.
[90] Li X, Chen Y, Wang J, Jiang C, Huang Y. Lung cancer cell-derived exosomal let-7d-5p down-regulates OPRM1 to promote cancer-induced bone pain. Front Cell Develop Biol. 2021;9:1–10.
[91] Liao Z, Ke W, Liu H, Tong B, Wang K, Feng X, Hua W, Wang B, Song Y, Luo R, Liang H, Zhang W, Zhao K, Li S, Yang C. Vasorin-containing small extracellular vesicles retard intervertebral disc degeneration utilizing an injectable thermoresponsive delivery system. J Nanobiotechnol 2022;20:420.
[92] Liu X-M, Ma L, Schekman R. Selective sorting of microRNAs into exosomes by phase-separated YBX1 condensates. eLife. 2021;10: 1–31.
[93] Lu A, Wawro P, Morgens DW, Portela F, Bassik MC, Pfeffer SR. Genome-wide interrogation of extracellular vesicle biology using barcoded miRNAs. eLife. 2018;7:1–23.
[94] Lu Y, Zhang J, Zeng F, Wang P, Guo X, Wang H, Qin Z, Tao T. Human PMSCs-derived small extracellular vesicles alleviate neuropathic pain through miR-26a-5p/Wnt5a in SNI mice model. J Neuroinflammation 2022;19:221.
[95] Luo X, Jean-Toussaint R, Sacan A, Ajit SK. Differential RNA packaging into small extracellular vesicles by neurons and astrocytes. Cell Commun Signal 2021;19:75.
[96] Ma L, Singh J, Schekman R. Two RNA-binding proteins mediate the sorting of miR223 from mitochondria into exosomes. Elife. 2023;12: 1–23.
[97] Malcangio M. Role of the immune system in neuropathic pain. Scand J Pain 2019;20:33–7.
[98] Mayor S, Parton RG, Donaldson JG. Clathrin-independent pathways of endocytosis. Cold Spring Harb Perspect Biol. 2014;6:1–22.
[99] McDonald MK, Ramanathan S, Touati A, Zhou Y, Thanawala RU, Alexander GM, Sacan A, Ajit SK. Regulation of proinflammatory genes by the circulating microRNA hsa-miR-939. Scientific Rep. 2016;6:1–11.
[100] McDonald MK, Tian Y, Qureshi RA, Gormley M, Ertel A, Gao R, Aradillas Lopez E, Alexander GM, Sacan A, Fortina P, Ajit SK. Functional significance of macrophage-derived exosomes in inflammation and pain. PAIN 2014;155:1527–39.
[101] McKenzie AJ, Hoshino D, Hong NH, Cha DJ, Franklin JL, Coffey RJ, Patton JG, Weaver AM. KRAS-MEK signaling controls Ago2 sorting into exosomes. Cell Rep 2016;15:978–87.
[102] Men Y, Yelick J, Jin S, Tian Y, Chiang MSR, Higashimori H, Brown E, Jarvis R, Yang Y. Exosome reporter mice reveal the involvement of exosomes in mediating neuron to astroglia communication in the CNS. Nat Commun 2019;10:4136.
[103] 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.
[104] Moen A, Jacobsen D, Phuyal S, Legfeldt A, Haugen F, Røe C, Gjerstad J. MicroRNA-223 demonstrated experimentally in exosome-like vesicles is associated with decreased risk of persistent pain after lumbar disc herniation. J Translational Med 2017;15:89.
[105] Mukherjee K, Ghoshal B, Ghosh S, Chakrabarty Y, Shwetha S, Das S, Bhattacharyya SN. Reversible HuR-microRNA binding controls extracellular export of miR-122 and augments stress response. EMBO Rep 2016;17:1184–203.
[106] Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles 2014;3.
[107] Murphy DE, de Jong OG, Brouwer M, Wood MJ, Lavieu G, Schiffelers RM, Vader P. Extracellular vesicle-based therapeutics: natural versus engineered targeting and trafficking. Exp Mol Med 2019;51:1–12.
[108] Mustonen AM, Palviainen M, Säisänen L, Karttunen L, Tollis S, Esrafilian A, Reijonen J, Julkunen P, Siljander PR, Kröger H, Mäki J, Arokoski J, Nieminen P. Tetraspanin profiles of serum extracellular vesicles reflect functional limitations and pain perception in knee osteoarthritis. Arthritis Res Ther 2024;26:33.
[109] Nieves Torres D, Lee SH. Inter-neuronal signaling mediated by small extracellular vesicles: wireless communication? Front Mol Neurosci. 2023;16:1–9.
[110] Orlova IA, Alexander GM, Qureshi RA, Sacan A, Graziano A, Barrett JE, Schwartzman RJ, Ajit SK. MicroRNA modulation in complex regional pain syndrome. J Transl Med 2011;9:195.
[111] Paba C, Dorigo V, Senigagliesi B, Tormena N, Parisse P, Voitchovsky K, Casalis L. Lipid bilayer fluidity and degree of order regulates small EVs adsorption on model cell membrane. J Colloid Interf Sci 2023;652: 1937–43.
[112] Parkitny L, McAuley JH, Di Pietro F, Stanton TR, O'Connell NE, Marinus J, van Hilten JJ, Moseley GL. Inflammation in complex regional pain syndrome: a systematic review and meta-analysis. Neurology 2013;80: 106–17.
[113] Peng T, Guo Y, Gan Z, Ling Y, Xiong J, Liang X, Cui J. Nerve growth factor (NGF) encourages the neuroinvasive potential of pancreatic cancer cells by activating the warburg effect and promoting tumor derived exosomal miRNA-21 expression. Oxidative Med Cell Longevity. 2022;2022:1–19.
[114] Pistono C, Bister N, Stanová I, Malm T. Glia-derived extracellular vesicles: role in central nervous system communication in health and disease. Front Cell Dev Biol. 2020;8:1–16.
[115] Polli A, Godderis L, Ghosh M, Ickmans K, Nijs J. Epigenetic and miRNA expression changes in people with pain: a systematic review. J Pain 2020;21:763–80.
[116] Price TJ, Rashid MH, Millecamps M, Sanoja R, Entrena JM, Cervero F. Decreased nociceptive sensitization in mice lacking the fragile X mental retardation protein: role of mGluR1/5 and mTOR. J Neurosci 2007;27: 13958–67.
[117] Putz U, Howitt J, Lackovic J, Foot N, Kumar S, Silke J, Tan SS. Nedd4 family-interacting protein 1 (Ndfip1) is required for the exosomal secretion of Nedd4 family proteins. J Biol Chem 2008;283:32621–7.
[118] Raiborg C, Stenmark H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature 2009;458:445–52.
[119] Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, Keefe FJ, Mogil JS, Ringkamp M, Sluka KA, Song XJ, Stevens B, Sullivan MD, Tutelman PR, Ushida T, Vader K. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. PAIN 2020;161:1976–82.
[120] Ramanathan S, Douglas SR, Alexander GM, Shenoda BB, Barrett JE, Aradillas E, Sacan A, Ajit SK. Exosome microRNA signatures in patients with complex regional pain syndrome undergoing plasma exchange. J Translational Med 2019;17:81.
[121] Ramanathan S, Shenoda BB, Lin Z, Alexander GM, Huppert A, Sacan A, Ajit SK. Inflammation potentiates miR-939 expression and packaging into small extracellular vesicles. J Extracell Vesicles. 2019;8:1–14.
[122] Raoof R, Martin Gil C, Lafeber F, de Visser H, Prado J, Versteeg S, Pascha MN, Heinemans ALP, Adolfs Y, Pasterkamp J, Wood JN, Mastbergen SC, Eijkelkamp N. Dorsal root ganglia macrophages maintain osteoarthritis pain. J Neurosci 2021;41:8249–61.
[123] 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 2014;1841:108–20.
[124] Record M, Silvente-Poirot S, Poirot M, Wakelam MJO. Extracellular vesicles: lipids as key components of their biogenesis and functions. J Lipid Res 2018;59:1316–24.
[125] Reinhold AK, Kindl GK, Dietz C, Scheu N, Mehling K, Brack A, Birklein F, Rittner HL. Molecular and clinical markers of pain relief in complex regional pain syndrome: an observational study. Eur J Pain 2023;27: 278–88.

[126] Rissland OS. The organization and regulation of mRNA–protein complexes. Wiley Interdiscip Rev. 2017;8:1–17.
[127] Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol 2014;14:195–208.
[128] Russell AE, Sneider A, Witwer KW, Bergese P, Bhattacharyya SN, Cocks A, Cocucci E, Erdbrügger U, Falcon-Perez JM, Freeman DW, Gallagher TM, Hu S, Huang Y, Jay SM, Kano SI, Lavieu G, Leszczynska A, Llorente AM, Lu Q, Mahairaki V, Muth DC, Noren Hooten N, Ostrowski M, Prada I, Sahoo S, Schøyen TH, Sheng L, Tesch D, Van Niel G, Vandenbroucke RE, Verweij FJ, Villar AV, Wauben M, Wehman AM, Yin H, Carter DRF, Vader P. Biological membranes in EV biogenesis, stability, uptake, and cargo transfer: an ISEV position paper arising from the ISEV membranes and EVs workshop. J Extracell Vesicles. 2019;8:1–33.
[129] Sabina S, Panico A, Mincarone P, Leo CG, Garbarino S, Grassi T, Bagordo F, De Donno A, Scoditti E, Tumolo MR. Expression and biological functions of miRNAs in chronic pain: a review on human studies. Int J Mol Sci. 2022;23:1–32.
[130] Salavessa L, Lagache T, Malardé V, Grassart A, Olivo-Marin JC, Canette A, Trichet M, Sansonetti PJ, Sauvonnet N. Cytokine receptor cluster size impacts its endocytosis and signaling. Proc Natl Acad Sci USA. 2021;118:1–12.
[131] Sanna MD, Quattrone A, Galeotti N. Silencing of the RNA-binding protein HuR attenuates hyperalgesia and motor disability in experimental autoimmune encephalomyelitis. Neuropharmacology 2017;123:116–25.
[132] Santangelo L, Giurato G, Cicchini C, Montaldo C, Mancone C, Tarallo R, Battistelli C, Alonzi T, Weisz A, Tripodi M. The RNA-binding protein SYNCRIP is a component of the hepatocyte exosomal machinery controlling MicroRNA sorting. Cell Rep 2016;17:799–808.
[133] Savina A, Furlán M, Vidal M, Colombo MI. Exosome release is regulated by a calcium-dependent mechanism in K562 cells. J Biol Chem 2003;278:20083–90.
[134] Schmidt O, Teis D. The ESCRT machinery. Curr Biol 2012;22:R116–20.
[135] Schnatz A, Müller C, Brahmer A, Krämer-Albers EM. Extracellular Vesicles in neural cell interaction and CNS homeostasis. FASEB Bioadv 2021;3:577–92.
[136] Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: novel pro-resolving lipid mediators in resolution. Semin Immunol 2015;27:200–15.
[137] Shurtleff MJ, Temoche-Diaz MM, Karfilis KV, Ri S, Schekman R. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. eLife. 2016;5:1–23.
[138] Simeoli R, Montague K, Jones HR, Castaldi L, Chambers D, Kelleher JH, Vacca V, Pitcher T, Grist J, Al-Ahdal H, Wong L-F, Perretti M, Lai J, Mouritzen P, Heppenstall P, Malcangio M. Exosomal cargo including microRNA regulates sensory neuron to macrophage communication after nerve trauma. Nat Commun 2017;8:1778.
[139] Sosanya NM, Kumar R, Clifford JL, Chavez R, Dimitrov G, Srinivasan S, Gautam A, Trevino AV, Williams M, Hammamieh R, Cheppudira BP, Christy RJ, Crimmins SL. Identifying plasma derived extracellular vesicle (EV) contained biomarkers in the development of chronic neuropathic pain. J Pain 2020;21:82–96.
[140] 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;8:1432–46.
[141] Srinivasan S, Yeri A, Cheah PS, Chung A, Danielson K, De Hoff P, Filant J, Laurent CD, Laurent LD, Magee R, Moeller C, Murthy VL, Nejad P, Paul A, Rigoutsos I, Rodosthenous R, Shah RV, Simonson B, To C, Wong D, Yan IK, Zhang X, Balaj L, Breakefield XO, Daaboul G, Gandhi R, Lapidus J, Londin E, Patel T, Raffai RL, Sood AK, Alexander RP, Das S, Laurent LC. Small RNA sequencing across diverse biofluids identifies optimal methods for exRNA isolation. Cell 2019;177:446–62.e16.
[142] Statello L, Maugeri M, Garre E, Nawaz M, Wahlgren J, Papadimitriou A, Lundqvist C, Lindfors L, Collen A, Sunnerhagen P, Ragusa M, Purrello M, Di Pietro C, Tigue N, Valadi H. Identification of RNA-binding proteins in exosomes capable of interacting with different types of RNA: RBP-facilitated transport of RNAs into exosomes. PLoS One. 2018;13:1–30.
[143] Stuffers S, Sem Wegner C, Stenmark H, Brech A. Multivesicular endosome biogenesis in the absence of ESCRTs. Traffic 2009;10:925–37.
[144] Sundararaman B, Zhan L, Blue SM, Stanton R, Elkins K, Olson S, Wei X, Van Nostrand EL, Pratt GA, Huelga SC, Smalec BM, Wang X, Hong EL, Davidson JM, Lécuyer E, Graveley BR, Yeo GW. Resources for the comprehensive discovery of functional RNA elements. Mol Cell 2016;61:903–13.
[145] Tafuri E, Santovito D, de Nardis V, Marcantonio P, Paganelli C, Affaitati G, Bucci M, Mezzetti A, Giamberardino MA, Cipollone F. MicroRNA profiling in migraine without aura: pilot study. Ann Med 2015;47:468–73.
[146] Takakura Y, Matsumoto A, Takahashi Y. Therapeutic application of small extracellular vesicles (sEVs): pharmaceutical and pharmacokinetic challenges. Biol Pharm Bull 2020;43:576–83.
[147] Tao X, Xue F, Xu J, Wang W. Platelet-rich plasma-derived extracellular vesicles inhibit NF-κB/NLRP3 pathway-mediated pyroptosis in intervertebral disc degeneration via the MALAT1/microRNA-217/SIRT1 axis. Cell Signal. 2024;117:1–14.
[148] Tavasolian F, Moghaddam AS, Rohani F, Abdollahi E, Janzamin E, Momtazi-Borojeni AA, Moallem SA, Jamialahmadi T, Sahebkar A. Exosomes: effectual players in rheumatoid arthritis. Autoimmun Rev. 2020;19:1–8.
[149] Teng Y, Ren Y, Hu X, Mu J, Samykutty A, Zhuang X, Deng Z, Kumar A, Zhang L, Merchant ML, Yan J, Miller DM, Zhang H-G. MVP-mediated exosomal sorting of miR-193a promotes colon cancer progression. Nat Commun. 2017;8:2–16.
[150] Tian T, Zhu YL, Hu FH, Wang YY, Huang NP, Xiao ZD. Dynamics of exosome internalization and trafficking. J Cell Physiol 2013;228:1487–95.
[151] Tkach M, Kowal J, Théry C. Why the need and how to approach the functional diversity of extracellular vesicles. Philos Trans R Soc Lond B Biol Sci. 2018;373:1–9.
[152] Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brügger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science 2008;319:1244–7.
[153] Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011;39:7223–33.
[154] 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;9:654–9.
[155] van der Vlist M, Raoof R, Willemen H, Prado J, Versteeg S, Martin Gil C, Vos M, Lokhorst RE, Pasterkamp RJ, Kojima T, Karasuyama H, Khoury-Hanold W, Meyaard L, Eijkelkamp N. Macrophages transfer mitochondria to sensory neurons to resolve inflammatory pain. Neuron 2022;110:613–26.e9.
[156] van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 2018;19:213–28.
[157] Vietri M, Radulovic M, Stenmark H. The many functions of ESCRTs. Nat Rev Mol Cell Biol 2020;21:25–42.
[158] Villarroya-Beltri C, Baixauli F, Gutierrez-Vazquez C, Sanchez-Madrid F, Mittelbrunn M. Sorting it out: regulation of exosome loading. Semin Cancer Biol 2014;28:3–13.
159. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez J, Martin-Cofreces N, Martinez-Herrera DJ, Pascual-Montano A, Mittelbrunn M, Sánchez-Madrid F. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun. 2013;4:1–10.
[160] Weaver JC. Electroporation: a general phenomenon for manipulating cells and tissues. J Cell Biochem 1993;51:426–35.
[161] Wei D, Zhan W, Gao Y, Huang L, Gong R, Wang W, Zhang R, Wu Y, Gao S, Kang T. RAB31 marks and controls an ESCRT-independent exosome pathway. Cell Res 2021;31:157–77.
[162] Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, Cai H, Di Vizio D, Driedonks TAP, Erdbrügger U, Falcon-Perez JM, Fu QL, Hill AF, Lenassi M, Lim SK, Mahoney MG, Mohanty S, Möller A, Nieuwland R, Ochiya T, Sahoo S, Torrecilhas AC, Zheng L, Zijlstra A, Abuelreich S, Bagabas R, Bergese P, Bridges EM, Brucale M, Burger D, Carney RP, Cocucci E, Crescitelli R, Hanser E, Harris AL, Haughey NJ, Hendrix A, Ivanov AR, Jovanovic-Talisman T, Kruh-Garcia NA, Ku'ulei-Lyn Faustino V, Kyburz D, Lässer C, Lennon KM, Lötvall J, Maddox AL, Martens-Uzunova ES, Mizenko RR, Newman LA, Ridolfi A, Rohde E, Rojalin T, Rowland A, Saftics A, Sandau US, Saugstad JA, Shekari F, Swift S, Ter-Ovanesyan D, Tosar JP, Useckaite Z, Valle F, Varga Z, van der Pol E, van Herwijnen MJC, Wauben MHM, Wehman AM, Williams S, Zendrini A, Zimmerman AJ. MISEV Consortium. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J Extracell Vesicles. 2024;13:1–84.
[163] Wen C, Seeger RC, Fabbri M, Wang L, Wayne AS, Jong AY. Biological roles and potential applications of immune cell-derived extracellular vesicles. J Extracell Vesicles. 2017;6:1–19.
[164] Wickman JR, Luo X, Li W, Jean-Toussaint R, Sahbaie P, Sacan A, Clark JD, Ajit SK. Circulating microRNAs from the mouse tibia fracture model

reflect the signature from patients with complex regional pain syndrome. Pain Rep 2021;6:e950.
[165] Wilczynska A, Bushell M. The complexity of miRNA-mediated repression. Cell Death Differ 2015;22:22–33.
[166] Wilson JE, Today BA, Salazar M, Kuo J, Ransom JT, Lightner AL, Chen G, Wong A. Safety of bone marrow derived mesenchymal stem cell extracellular vesicle injection for lumbar facet joint pain. Regen Med 2024;19:19–26.
[167] Woodman PG, Futter CE. Multivesicular bodies: co-ordinated progression to maturity. Curr Opin Cell Biol 2008;20:408–14.
[168] Wozniak AL, Adams A, King KE, Dunn W, Christenson LK, Hung W-T, Weinman SA. The RNA binding protein FMR1 controls selective exosomal miRNA cargo loading during inflammation. J Cell Biol. 2020;219:1–13.
[169] Wu X, Wang L, Cong M, Shen M, He Q, Ding F, Shi H. Extracellular vesicles from skin precursor-derived Schwann cells promote axonal outgrowth and regeneration of motoneurons via Akt/mTOR/p70S6K pathway. Ann Translat Med 2020;8:1640.
[170] Yang H-y, Vonk LA, Licht R, van Boxtel AMG, Bekkers JEJ, Kragten AHM, Hein S, Varghese OP, Howard KA, Öner FC, Dhert WJA, Creemers LB. Cell type and transfection reagent-dependent effects on viability, cell content, cell cycle and inflammation of RNAi in human primary mesenchymal cells. Eur J Pharm Sci 2014;53:35–44.
[171] Zhang C, Huang Y, Ouyang F, Su M, Li W, Chen J, Xiao H, Zhou X, Liu B. Extracellular vesicles derived from mesenchymal stem cells alleviate neuroinflammation and mechanical allodynia in interstitial cystitis rats by inhibiting NLRP3 inflammasome activation. J Neuroinflammation 2022; 19:80.
[172] Zhang K, Li P, Jia Y, Liu M, Jiang J. Concise review: current understanding of extracellular vesicles to treat neuropathic pain. Front Aging Neurosci. 2023;15:1–10.
[173] Zhang L, Liu J, Zhou C. Current aspects of small extracellular vesicles in pain process and relief. Biomater Res 2023;27:78.
174. Zhang X, Zhu L, Wang X, Xia L, Zhang Y. Advances in the role and mechanism of miRNA in inflammatory pain. Biomed Pharmacother. 2023;161:1–9.
175. Zhao YY, Wu ZJ, Zhu LJ, Niu TX, Liu B, Li J. Emerging roles of miRNAs in neuropathic pain: from new findings to novel mechanisms. Front Mol Neurosci. 2023;16:1–16.
[176] Zhou X, Brown BA, Siegel AP, El Masry MS, Zeng X, Song W, Das A, Khandelwal P, Clark A, Singh K, Guda PR, Gorain M, Timsina L, Xuan Y, Jacobson SC, Novotny MV, Roy S, Agarwal M, Lee RJ, Sen CK, Clemmer DE, Ghatak S. Exosome-mediated crosstalk between keratinocytes and macrophages in cutaneous wound healing. ACS Nano 2020;14:12732–48.
[177] Zhu H, Guariglia S, Yu RYL, Li W, Brancho D, Peinado H, Lyden D, Salzer J, Bennett C, Chow C-W. Mutation of SIMPLE in Charcot–Marie–Tooth 1C alters production of exosomes. Mol Biol Cell 2013;24:1619.