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Exosomes as a new pain biomarker opportunity

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Abstract

Exosomes are extracellular microvesicles implicated in intercellular communication with ability to transfer cargo molecules, including protein, lipids, and nucleic acids, at both close and distant target sites. It has been shown that exosomes are implicated in physiological and pathological processes. In recent years, the interest on exosomes' role in many pain states has increased. Their involvements in pain processes have been demonstrated by studies on different chronic pain diseases, both inflammatory and neuropathic, such as osteoarthritis, rheumatoid arthritis, inflammatory bowel diseases, neurodegenerative pathologies, complex regional pain syndrome, and peripheral nerve injury. Animal and clinical studies investigated exosomes-based treatments, showing their ability to improve painful symptoms with fewer side effects, with potential immunoprotective and anti-inflammatory effect. Specific molecular patterns characterize exosomes' cargo according to the cellular origin, epigenetic modifications, environmental state, and stressor factors. Therefore, the identification of specific cargo's profile associated to pain states may lead to recognize specific pathological states and to consider the use of exosomes as biomarkers of diseases. Furthermore, exosomes' ability to transfer information and their presence in many accessible biological fluids suggest a potential use as novel non-invasive therapeutic tools in pain field.

Keywords

Exosomes, extracellular microvesicles, exosomes' cargo, pain, biomarkers, therapeutic tool

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Introduction

Chronic pain is one of the most common worldwide problem, which affects 20% to 25% of people, especially older than 65 years.[1] In recent years, the interest in extracellular circulation vesicles and their role in different pain states are increased, and the most attention among them was attracted by exosomes. Exosomes are nanosized vesicles of endosomal origin fused with the plasma membrane. They have been demonstrated to mediate the transfer of cargo including proteins, lipids, and nucleic acids into the extracellular environment or in target cells.[2] The fact that exosomes have been detected in many body fluids (e.g. blood,[3] cerebrospinal fluid (CSF),[4] urine,[5] sperm,[6] breast milk,[7] and nasal secretions[8]) highlights their role as intercellular communication mediators and, thanks to their transfer ability, they are involved in both pathological and physiological processes. Exosomes' involvement in pain processes have been demonstrated by studies on different chronic pain diseases, in particular osteoarthritis (OA),[9,10] rheumatoid arthritis (RA),[11] inflammatory bowel diseases (IBDs),[12] neurodegenerative pathologies,[13] and complex regional pain syndrome (CRPS).[14] From some of these studies, exosomes appear to be a possible novel therapeutic strategy,[15] transferring cargo in close and distance target cells. They have been even identified as a promising treatment able to improve painful symptoms with fewer side effects,[15] with potential immunoprotective[16] and anti-inflammatory role.[10] Moreover, it has been

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found that exosomes might represent a tool to distinguish subgroups of patients with higher possibility to benefit from specific treatments.[14] In this narrative review, we provide an overview of exosomes in pain field present in literature. Possible associations between exosomes and painful diseases are discussed. The aims are to discuss the physiological role of exosomes for potential use as (1) biomarkers and/or (2) novel therapeutic tools.

Physiological role of exosomes

Exosomes are the smallest extracellular vesicles, 30 to 100 nm in size, released by various cell types and generated by the inward budding of multivesicular endosomes membrane, containing intraluminal vesicles (ILVs), to the plasma membrane.[17] This fusion requires the involvement of small GTPases of the Rab family and induces the release of the content including ILVs; once shed, the ILVs are called exosomes. Compared to other extracellular vesicles, such as microvesicles or ectosomes, exosomes differ in size, lipids composition, content, and cellular origin. By transmission electron microscopy, it has been suggested that exosomes have a cup morphology and a bilayer lipid structure.[18] The bilayer membrane confers stability to the structure, protects cargo by degradation processes, and allows exosomes' moving across biological barriers, thanks to membrane adhesive proteins.[19]

Exosomes have a specific gene expression profile by which it is possible to detect and differentiate them from other extracellular vesicles. In particular, this specific protein pattern includes tetraspanins (CD9, CD63, CD81, and CD82), heat shock proteins (HSP60, HSP70, and HSP90), tumor susceptibility gene 101 protein (TSG101), and ALG-2-interacting protein X (ALIX).[20] Their biogenesis within a cell seems to be mediated by a complex composed of four multiprotein sub-complexes that also guides the sorting of the intracellular cargo, the Endosomal Sorting Complex Required for Transport (ESCRT).[21] The intercellular communication seems to involve two different mechanisms: the first one precisely based on ESCRT machinery in combination with other players, such as syndacans transmembrane protein, cytosolic adaptor syntenin, and the accessory protein ALIX.[22] Syndecans with their heparan sulphate polysaccharide chains mediate many of surface signaling events, connecting with cytosolic adaptor syntenin, that in its turn interacts directly with ALIX.[23] ALIX binds ESCRT-III, one of multiprotein sub-complex, allowing the intraluminal budding of endosomal membranes.[22]

The second method of intercellular communication proposed is an alternative ESCRT-independent mechanisms that involve tetraspanins and lipids to mediate the sorting of specific molecules into exosomes.[24]

These membrane vesicles are present in numerous biological fluids[3–8] and different conditions can induce their release, both physiologically and pathologically. Their cargo is characterized by a broad range of molecules including proteins, messenger RNA, long non-coding RNA, circular RNA, DNA, and lipids,[24] which depend on cellular origin and enviromental state. The protein composition of exosomes enables to get rid of obsolete and toxic material; in fact, many misfolded and prion proteins involved in the development of neurodegenerative diseases are released and exported through exosomes.[25] Therefore, these proteins have a quality control role and are involved in preservation of homeostasis. Similarly, exosomes participate in the regulation of intracellular RNA homeostasis by promoting the release of misfolded or degraded RNA products.[26] Exosomes are implicated in the immune response carrying, for example, antigens from the cells from which they originate.[20] In particular, unidirectional transfer of micro RNA (miRNA) from the T cell to the antigen-presenting cells was demonstrated.[27] They have been shown to induce targeted endothelial cell migration through the enhanced secretion of monocytic miR-150,[28] highlighting their role affecting recipient cells function. The exosome-mediated transfer of RNA to silence target genes between cells was demonstrated.[29] Exosomes are also involved in lipid homeostasis supporting the low-density lipoprotein release, as seen in Niemann-Pick type C disease.[30]

Exosomes in different chronic pain conditions

Chronic pain is a complex multifactorial disease with a serious impact on the quality of life due to a variety of associated symptoms such as anxiety, depression, insomnia, and other mood disorders. The lack of a precise understanding of its pathophysiology, early diagnosis interventions, and effective treatments can result in a significant morbidity and mortality.[31]

Nowadays, the available treatments are based on the use of opioids and nonsteroidal anti-inflammatory drugs,[32] addressed to reduce symptoms without complete recovery, often causing side effects such as constipation, addiction, impairment of immune system, and opioid-induce hyperalgesia.[33] Furthermore, there is an interindividual variability on therapy's response, with relevant implications in terms of efficacy and safety.[34] Research on both potential treatments and early diagnosis biomarkers is essential, in light of the urgent need to minimize the severe effects of chronic pain in individuals and in the society. Many researchers are involved in

biomarkers discovery in pain field and many data have been obtained at "-omics" level (glycomics, Activomics, genome-wide association studies, and epigenomics),[35,36] but other studies are needed to be validated and to be transferred in clinical practice. Because of exosomes' ability to transfer information from an origin cell to a recipient cell[2] and their presence in many accessible biological fluids,[3–8] they may reveal novel and promising insights in the pain field. In the last years, exosomes have been increasingly found associated with pain conditions. An overview of the studies investigating exosomes in neuropathic and inflammatory chronic pain conditions is reported in Table 1.

Exosome-based treatments

Exosomes of mesenchymal stem cells (MSCs) have been shown to relieve painful symptoms, with an analgesic action in several chronic pain models and fewer side effects.[15] The use of MSCs has been suggested as a treatment for peripheral nerve injury,[19] but their therapeutically effects are linked to their exosomes which can transfer miRNAs cargo to target neurons, in order to favorite axonal growth and neural survival.[37] The mechanisms with which these exosomes act are until now unclear, but the demonstrated presence of many neurotrophic factors between MSC-exosomes' cargos, such as glial cell-derived neurotrophic factor (GDNF), fibroblast growth factor-1 (FGF-1), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IFG-1) and nerve growth factor (NGF), supports the idea of their potential efficacy in peripheral nerve injury treatment.[19] Furthermore, Simeoli et al. have suggested the use of miR-21-5p antagomir to regulate the iperexpression of miR-21-5p found in neuronal exosomes of dorsal root ganglia, after nerve injury in mice. Overexpression of MiR-21-5p supports proinflammatory phenotype of macrophages attracted on the site of damage, while intrathecal delivery of a miR-21-5p antagomir appears to avoid an extension of inflammatory condition and neuropathic hypersensitivity onset.[38]

Exosomes appear to be involved in immune regulation and antigen presentation,[39,40] thanks to presence among their cargos of immunomodulatory mediators such as transforming growth factor, interferon-γ, indoleamine 2,3-dioxygenase, prostaglandin E_2 (PGE_2), heme oxygenase-1, and interleukin-10 (IL-10).[41]Macrophage-derived exosomes have been shown to have an immunosuppressive role; in particular, Jiang et al. demonstrated that the transfer of exosomes produced by intestinal epithelial cells into IBD mice could induce regulatory T cells and immunosuppressive dendritic cells and subsequent decrease the severity of the disease.[12]

Exosomes derived from immune cells or nervous cells also have the ability to cross blood–brain barrier with neuroprotective and tissue repair effects.[42] This ability suggests the possibility to use exosomes to transfer specific drug molecules acting in target tissue to resolve cerebral injury.[43] Many studies have been conducted to verify the exosomes' capacity to cross blood–brain barrier such as Alvarez-Erviti et al. who demonstrated that small interfering RNAs mediated by exosomes of dendritic cells interfered in the Alzheimer's treatment in mouse brain model. Then, it could be explained with exosomes' ability to passes the barrier and delivers their cargos to target cells.[44]

Exosome-based biomarker diagnosis

Exosomes could represent novel non-invasive biomarkers of specific pain diseases in order to obtain an early diagnosis. The molecular pattern inside exosomes is different according to the cellular origin, epigenetic modifications, environmental state, and stressor factors. Therefore, identification of specific cargo's profile associated to pain states might lead to distinguish a specific pathological state from a healthy state and to use exosomes as biomarkers of the diseases. Human studies on CRPS identified specific miRNAs that could be useful to identify subgroup of patients in order to find personalized therapeutic strategies,[14] for example, low level of miR-338-5p in CRPS patients could identify poor responders to plasmapheresis treatment; it could suggest clinicians to evaluate alternative strategies.[45] Moreover, in IBD patients' exosomes, an overexpression of annexine-1 during inflammatory process than healthy controls has been found, highlighting its potential role as diagnosis biomarker.[46]

Thanks to their capability to transfer miRNAs, exosomes also have an important role in neuronal mechanisms such as synaptic plasticity, neurogenesis, and neuronal differentiation.[47] About their involvement in neurodegenerative diseases,[13] often the research of biomarkers is conducted on blood sample, thanks to its easy collection protocol without risks of an invasive procedure; however, some limits about blood biomarkers utility were found.[48] One of them in particular is that blood biomarkers are not exclusively central nervous system (CNS)-specific and, then, they could be impaired with their diagnostic and prognostic values. On the contrary, CSF sample collection is an invasive procedure with a lumbar puncture, but as CSF is directly connected with brain and spinal cord, possible molecular changes in their microenvironments are highlighted by its composition, making it a better source for biomarkers research of neuronal diseases than blood.[49]

Table 1. Overview of studies investigating exosomes role in neuropathic and inflammatory chronic pain conditions.

	Specific conditions	Samples	Main findings	PMID
Chronic Neuropathic pain	Spinal cord injury	Mouse SCI model	Ccl3 transfer from Schwann cells to blood through exosomes.	29164149
	Peripheral neuropathies	Rat Schwann cell exosomes and human serum	p75 and NCAM subtype-specifically expressed in the sera of patients with peripheral neuropathy.	31353867
	Sciatic nerve injury	Mice model, chronic constriction injury of the sciatic nerve	Release of exosomes from mPFC and NAc has elevated the pain sensations in the subjected mice.	31981720
	Sciatic nerve injury	Mice model, partial sciatic nerve ligation	Expression level of miR-21 significantly increased in exosomes extracted from blood of nerve-ligated mice	26990296
	Nerve injury	Mouse model induced by SNI	Upregulation of C5a and ICAM-1 in exosomes from SNI model compared to control.	31669360
	Peripheral axon injury	Mice, exosomal fraction of cultured DRG	Neuron–macrophage communication proposed as analgesic strategy and miR-21-5p proposed as specific target of the exosome cargo.	29176651
	Complex regional pain syndrome	Human subjects	hsa-miR-223- 5p increased in plasma exosomes in subjects with fracture with normal healing compared to subjects developing CRPS and healthy controls.	31095096
		Human subjects; HEK293 cells	17 miRNAs identified differentially expressed before and after therapy in CRPS patients; hsa-miR-338-5p regulates IL-6 mRNA and protein levels in vitro.	30871575
		HEK293 cells	Gene expression changes in recipient cells following the uptake of exosomes enriched in miR-939.	31489147
Chronic Inflammatory Pain	Rheumatoid arthritis	Synovial exosomes from patients with RA	Synovial exosomes contain citrullinated proteins	17133577
		Synovial fluid from patients with RA and OA	Plasma miRNAs had distinct patterns from synovial fluid miRNAs	20470394
		Human mesenchymal stem cells	Role of exosomes derived from MSCs overexpressing miR-92a-3p in enhancing chondrogenesis and suppressing cartilage degradation, through a mechanism involving a Wnt protein.	30257711
	Osteoarthritis	Human mesenchymal stem cells and rat model	Exosomes derived from MSCs transfected with miR-140-5p enhanced the proliferation and migration of articular chondrocytes and successfully prevented OA.	28042326
		Human mesenchymal stem cells	The lncRNA-KLF3-AS1 derived from MSCs induces chondrocyte proliferation and inhibits chondrocyte apoptosis.	30324848
		Human adipose stem cell on chondrocytes	Exosomes derived from adipose MSC reduce hypertrophy and dedifferentiation of chondrocytes, decreasing inflammatory mediators production, such as TNF-α, IL-6, PGE_2, and NO, and an increasing anti-inflammatory cytokine IL-10	23811540

(continued)

Table 1. Continued.

Specific conditions	Samples	Main findings	PMID
	Mesenchymal stem cell from adipose tissue from healthy individuals, Chondrocytes from OA patients	Exosomes from adipose MSC in OA chondrocytes decrease MMP-13 expression and enhance IL-10 and collagen II expression.	29763932
Inflammatory bowel disease	IBD patients' serum	An increased number of exosomes containing ANXA-1 was found.	25664854
	Rat Intestinal epithelial cells of IBD model	Exosomes from intestinal epithelial cells participate in maintaining of intestinal tract immune of homeostasis.	27721471
Neuroinflam-matory diseases	Human postmortem prefrontal cortices	Toxic forms of aggregated proteins have been found in brain exomes	24694258

SCI: spinal cord injury; SNI: spared nerve injury; NCAM: neural cell adhesion molecule; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; ICAM-1: intercellular Adhesion Molecule 1; DRG: dorsal root ganglia; CRPS: complex regional pain syndrome; RA: rheumatoid arthritis; OA: osteoarthritis; miRNA: micro RNA; MSCs: mesenchymal stem cells; TNF: tumor necrosis factor ; IL: interleukin; PGE_2: prostaglandin E_2; NO: nitric oxide; MMP-13: matrix metallopeptidase 13; IBD: inflammatory bowel diseases.

Exosomes role in neuropathic pain

Exosomes are released and retaken by neurons depending on synaptic activity, and the reported evidence on their inter-neuronal communication highlights the importance to look deeper into the perspectives of exosomes biomarkers in neuropathic pain states. For this purpose, many animal studies have been conducted. It has been demonstrated that Ccl3, a chemokine potentially mediating peripheral and central sensitization in neuropathic pain, was transferred from Schwann cells to peripheral blood via exosomes.[43] Moreover, proteomics analysis revealed p75 and neural cell adhesion molecule (NCAM) exosomes proteins as potential human serum biomarkers reflecting alterations in Schwann cells in inflammatory and inherited neuropathy.[50] In a sciatic nerve mouse model, exosomes were also quantified over time in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc), revealing that their release can mimic pain sensation-like behaviors; however, the projections from the mPFC to the NAc are important players in the reward circuitry and their activation inhibits pain behaviors. Exosomes release from these brain areas were proposed to mediate pain threshold and allodynia.[51] A proteome characterization of exosomes from mouse spared nerve injury (SNI) model suggested the cargo sorting of vesicular proteins as a crucial step in mediating signaling mechanisms underlying neuropathic pain and evidenced unique patterns of proteins. In particular, significant upregulation of complement component 5a (C5a) and Intercellular Adhesion Molecule 1 are detected in exosomes from SNI model compared to sham control.[52] The involvement of exosomes in neuropathic pain is also underlined by many studies on CRPS. It is a chronic neuropathic pain disorder, disabling for sensory, motor, and autonomic dysfunctions as well as of allodynia, hyperalgesia, dystonia, and tremors.[53] Ramanathan et al. have identified a different miRNA–exosomal profile between responders and non-responders to treatment in CRPS patients, suggesting a potential tool to prior identify a subgroup of patients with higher possibility to have benefit by that specific treatment, in this case from plasmapheresis.[14] In a mouse model of CRPS, the mechanism of action of macrophage-derived exosomes and their cargo has been investigated. A decrement of thermal hyperalgesia following a single injection of macrophage-derived exosomes has been found, suggesting a potential immunoprotective role. In the same study, serum-derived exosomes from CRPS patients were analyzed and 127 miRNAs were significantly different comparing CRPS exosomes with control-derived exosomes. Among them, three miRNAs (miR-21-3p, miR-146a, and miR-146b), known to be involved in the control of over activation of innate immune response, are over expressed in both murine and human model.[16]

Exosomes role in inflammation and pain

Inflammation is an immune response against infection or injury that acts to restore tissue homeostasis. Homeostasis is re-established temporally and spatially through proinflammatory response to noxious conditions and protective mechanisms driving resolution of inflammation. However, uncontrolled or unresolved inflammation can be active pathways of systemic inflammation involved in the pathogenesis of several pain diseases such OA, RA, IBDs, and neurodegenerative

diseases. Exosomes are involved in many inflammatory diseases due to their capabilities to transfer different molecules as miRNAs and proteins acting on close or distant target tissues.[54] The association between inflammation and the levels of specific exosomal cargo molecules is a crucial step in the identification of possible novel biomarkers of inflammatory-based diseases. For example, RA is a chronic, inflammatory, and systemic autoimmune disease caused by inflammation of the synovium and patients are often affected by chronic pain due to long-term joint injury.[11] The onset of this pathology is not completely clarified, but exosomes involvement has been hypothesized as a mediating mechanism based on the evidence that citrullinate proteins were found in synovial exosomes isolated from a RA patients. Citrullination is a post translational change essential during the conversion process of non-immunogenic to auto-immunogenic proteins.[55] Exosomes were also demonstrated to exert an anti-inflammatory action. Indeed, exosomes derived from adipose MSCs have a role on chondro-protective and anti-inflammatory activities in OA.[9] The action of MSCs has been shown to trigger a decrease of inflammatory mediators production, such as tumor necrosis factor α, IL-6, PGE_2, and NO, and an increase of anti-inflammatory cytokine IL-10 in the chondrocytes.[56] Recent clinical trials showed a decrement of pain, an enhancement of joint performance, and a high quality of cartilage repair in knee OA, following MSC treatment.[57] Exosomes were also shown to be involved in the IBDs, which are a set of chronic disorders that occur when intestinal homeostasis is impaired.[12] One of the many mechanisms underlying these diseases is represented by the immune response modulated by macrophage activity. Macrophage-derived exosomes are involved in this pathophysiological mechanism through an immunosuppressive role. Indeed, it has been demonstrated that exosomes of normal intestine transferred into IBD mice are responsible of decrement of severity of disease.[12] Moreover, in IBD patients' serum, an increased number of exosomes containing annexine-1 was found. This protein has a key role in repairing of mucosal damage and is overexpressed during inflammatory process, representing a potential biomarker of disease.[46]

Finally, exosomes are involved in both physiological brain function and neuroinflammatory mechanisms. Neuroinflammation is characterized by a high level of pro-inflammatory cytokine production and glial cell activation, causing many neurodegenerative diseases including Parkinson's, Alzheimer's, and Creutzfeldt–Jacob diseases. Exosomes transfer to neighboring cells inflammatory molecules such as α-synuclein, amyloid β, and prions promoting the dissemination of the disease.[13] For the first time, the presence of miRNAs in CSF exosomes has been found by Gui et al. in Parkinson's and Alzheimer's patients.[58] Their results have shown a different expression of miRNAs between pathological condition and its healthy control and between both pathological conditions too, suggesting the possibility to use them like biomarkers to differential diagnosis and/or monitoring disease progression. Moreover, several studies on CSF have found differences in the levels of some inflammatory markers between neuropathic pain patients and healthy controls.[59,60]

Conclusions and future perspectives

The high socio-economical costs related to chronic pain are partly due to the lack of understanding of the underlying pathophysiology and of effective and safe treatments. Exosome microvesicles are found associated to physiological and pathological processes, involved in many chronic pain diseases, i.e. inflammatory and neuropathic pain. Thanks to their key role in intracellular communication and their transfer ability of molecules cargo to target cells, exosomes are considered to be promising novel non-invasive biomarkers for mechanisms underlying chronic pain. They can be collected from different body fluids (e.g. blood and CSF) as diagnostic biomarkers for various chronic pain states and may contribute to the development of new innovative pain management opportunities. In fact, in peripheral nerve injury treatment, it has been shown that the use of MCS–exosomes could avoid risks caused by MSC transplantation, like for example immune rejection, thanks to their low immunogenicity. [61] Furthermore, exosomes can cross biological barriers, thanks to their membranes composition,[19] and then they can be useful like carriers for transporting drug or biological peptide to regulate biological function of target cells. However, until now, an effective use in clinical practice is missing due to the absence of standardized protocol for the isolation and purification of exosomes, the long time needed to ending it and the expensive costs of labor procedures.[62] In addition, many studies show the involvement of exosomes in many pain processes but the molecular mechanism with which they act is until now not completely known. Therefore, further studies with large sample size are needed to fill these gaps and to obtain a full knowledge of exosomes value and to develop a valid, standardized, and cheap laboratory protocol. This is necessary to exosomes' application in clinical practice in order to go toward a precision medicine, patient-centered, with a revolutionary impact in terms of effectiveness, safety, ethics, and cost savings.

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