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Review

Exosomes in inflammation and role as biomarkers

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ABSTRACT

Exosomes are endosomal-derived nano-vesicles. They are considered vehicles through which donor cells transfer proteins, lipids and nucleic acids to target cells thus influencing their metabolism. Exosomes are involved in inflammatory processes that play a pivotal role in a large number of pathologic states including cancer, inflammatory bowel diseases, type 2 diabetes, obesity, rheumatoid arthritis and neurodegenerative diseases. The association between inflammation and change in nature or expression level of some exosomal cargos is the fundamental step for identifying possible novel biomarkers of inflammatory-based diseases. A novel interesting exosome cargo is the SLC22A5 transport protein whose level in exosomes is regulated by the pro-inflammatory cytokine INF-γ. The advantage of using exosomes as a biomarker vehicle consists of their ease of collection from body fluids such as urine and saliva as they may represent a non-invasive means for screening human pathology.

1. Introduction

1.1. Discovery of exosomes

Although the first study suggesting the existence of extracellular vesicles was carried out in 1946 [1], it was only in 1977 that De Broe described the release of these “membrane fragments” as a ubiquitous feature of viable cells. Until the 1980s, these “fragments” were, however, commonly considered as platelet “dust” or cellular debris directly budded from the plasma membrane [2]. It was only later that two independent groups demonstrated the existence of a subtype of vesicles having an intracellular origin. Rose Johnstone, the first researcher who isolated endosome-derived vesicles, proposed to call them exosomes [3]. Subsequent studies have shown that the biogenesis of exosomes is linked to the formation of endosomal multivesicular bodies which fuse with the plasma membrane and release these luminal vesicular bodies into the extracellular environment. This process can occur through two different mechanisms, the first involves the Endosomal Sorting Complex Required for Transport (ESCRT) machinery [4] and the second is ESCRT-independent involveing tetraspanins [5]. Unfortunately, the stimulus that may lead to the exocytosis of exosomes is still largely unexplained.

1.2. Physicochemical characteristics of exosomes

Exosomes originate from endosomal vesicles, a unique feature that distinguishes them from the other vesicles released by cells. Indeed, microvesicles are generated by budding of plasma membrane while apoptotic bodies are released from blebs of cells undergoing apoptosis [6]. From a physicochemical point of view, exosomes appear as cup-shaped vesicles by Transmission Electron Microscopy (TEM), and are 20–100 nm in size with a density of 1.10 g × mL^{-1} [7]. In contrast, microvesicles and apoptotic bodies show a heterogeneous morphology and their size range from 100 to 1000 nm and from 1 to 5 μm, respectively [6].

Abbreviations: ESCRT, Endosomal Sorting Complex Required for Transport; TEM, Transmission Electron Microscopy; TSG101, Tumour susceptibility gene 101 protein; CHMP2A, Charged multivesicular body protein 2a; RAB11B, Ras-related protein Rab-11B; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL-6, Interleukin 6; TNFα, Tumour Necrosis Factor alpha; GCSF, Granulocyte Colony-Stimulating Factor; CCL2, Chemokine C-C motif Ligand 2; MIF, Macrophage Migration Inhibitory Factor; PDAC, Pancreatic ductal adenocarcinoma; IBDs, Inflammatory bowel diseases; PSMA7, Proteasome Subunit Alpha type 7; TECs, tubular epithelial cells; ATF3, activating transcriptional factor 3; CPT1, Carnitine Palmitoyltransferase 1; CAC, Carnitine/Acylcarnitine Carrier; CPT2, Carnitine Palmitoyltransferase 2; CRP, C-Reactive Protein; SAA, Serum Amyloid A; INF-γ, Interferon gamma; TGF-β, Transforming growth factor beta; TLR4, Toll-like receptor 4; TRIF, TIR domain-containing adaptor protein inducing interferon beta; ADSC, Adipose-derived stem cells; RA, Rheumatoid Arthritis; AKT, Protein Kinase; AD, Alzheimer's disease; PD, Parkinson's disease; NEM, N-Ethylmaleimide; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; STAT1, STAT3, STAT5, Signal transducer and activator of transcription 1, 3, 5

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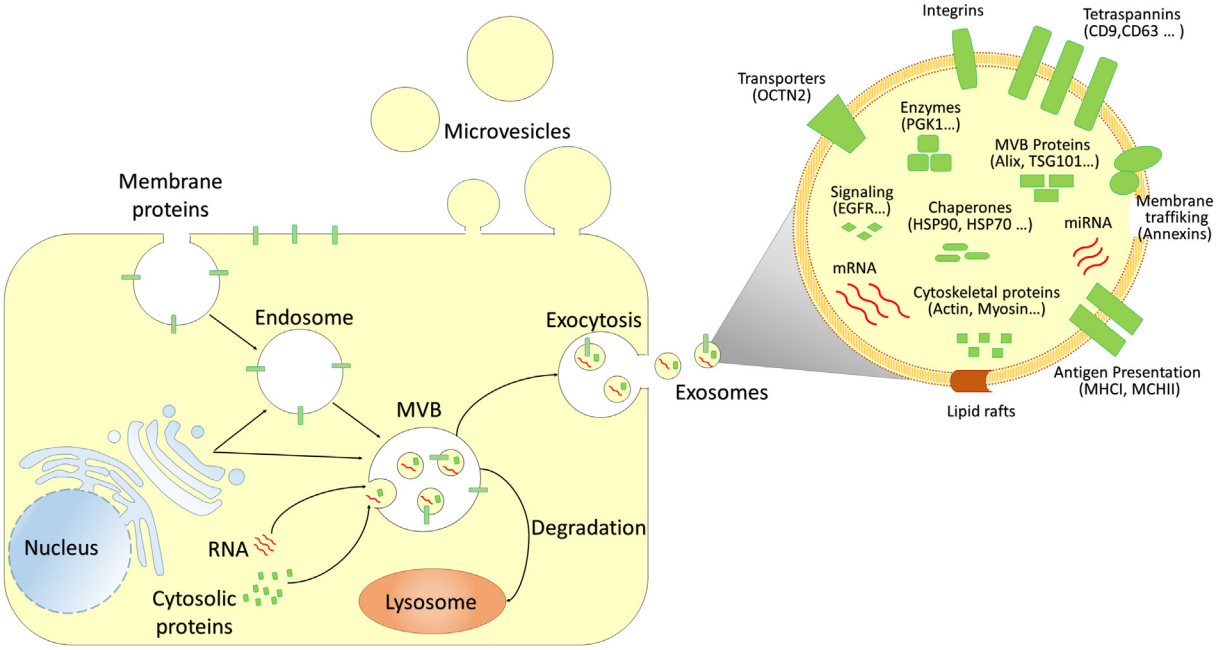

Fig. 1. Origin and molecular components of exosomes. Early endosomes evolve into late endosomes leading to the formation of the Multi-Vesicular Bodies (MVB). MVBs can fuse with lysosomes or with the plasma membrane, releasing their content as exosomes. The right panel shows an enlarged view of exosomes with a schematic representation of their components.

Table 1
Source and features of exosomes in some inflammatory-based diseases

Disease	Exosomes source	Role of exosomes	Ref.
Cancer	Gastric cancer cells	Stimulation of the NF-κB pathway in macrophages leading cell proliferation	[21]
	Breast cancer cells	The NF-κB signalling pathway is stimulated by exosomal palmitoylated proteins	[22]
	Several cancer cell lines	Exosomal miRNAs trigger pro-metastatic inflammatory response mediated by Toll-like receptor	[23]
	Pancreatic ductal adenocarcinoma	Exosomal MIF is a prognostic marker for PDAC liver metastasis	[24]
	prostate cancer cells LNCaP	Inhibition of T-cell proliferation and promotion of T-cell apoptosis through the FasL pathway	[26]
	Head and Neck squamous carcinoma cells	Tumour escape	[27]
	Nasopharyngeal carcinoma cells	Inhibition of T cell proliferation by modulation of ERK, STAT1, STAT3, STAT5 and MARK1 pathway.	[28]
Inflammatory Bowel Diseases	Normal intestine	Attenuation of IBD in mice	[32]
	Colonic luminal fluid aspirates	Exosomes from IBD patients increase IL-8 level in human colonocytes	[33]
	Intestinal epithelial cells	Patients with IBD have an elevated number of exosomes containing annexin-1	[36]
	Saliva	Marked differences in PSMA7 expression in exosome from IBD patients compared to controls	[37]
Kidney diseases	Tubular epithelial cells	During kidney injury, urinary exosomes contain mRNA coding for inflammatory chemokine	[39]
	Urine	mRNA of ATF3 is 60-fold higher in urinary exosomes from patients with acute kidney injury than in controls	[40]
	HEK293/urine	INF-γ cell treatment increases the level of OCTN2 in exosomes	[51]
Obesity and Diabetes	Adipose tissue	Exosomes from obese subjects play a role in the development of chronic inflammation by TGF-β and Wnt/β-catenin signalling	[53]
	Adipose tissue	Exosomes from obese mice modulate TLR4/TRIF pathway and are involved in obesity-associated insulin resistance	[54]
	Macrophages	Exosomes from macrophages of obese contain miRNAs which induce glucose intolerance	[55]
	Adipose-derived stem cells	Improvement of insulin sensitivity	[56]
Rheumatoid arthritis	Synovial fibroblasts	Vesicles from synovial fibroblasts treated with Interleukin-1β induce osteoarthritis-like phenotype	[57]
	Synovial fibroblasts	Exosomes from RA cause AKT and NF-kappa B activation. The activated T cells become resistant to apoptosis	[58]
	Synovial fluid	Exosomes from RA patient contain citrullinated protein	[59]
	Mesenchymal stem cells	Protect cartilage from degradation and promote its repair	[60,61]
Neuro-inflammation	Several cell lines	α-synuclein, amyloid β and prions are moved between cells into exosomes triggering an inflammatory cascade	[63]
	Serum	Exosomes from LPS-treated mice induce systemic and CNS inflammation	[62]
	Microglial cells	After brain injury the level of miR-124-3p increases causing the reduction of inflammation of neurons	[25]

1.3. Exosome composition

The interest in exosomes has progressively grown since they were discovered to contain functional protein, mRNA and microRNA [8,9]. Proteomic studies revealed that exosomes contain proteins originally located in endosomes, plasma membrane and cytosol. They contain very few components from the nucleus, mitochondria, endoplasmic reticulum or Golgi, confirming that their cargo is not random as might be in the case of cell debris. The protein content of exosome can be divided into two sets. The nature of proteins that belong to the first set depends on the cell type that secretes the exosomes, whereas proteins belonging to the second set are found in most exosomes and subsequently used as exosomal markers. Tumour Susceptibility Gene 101 protein (TSG101), Charged Multivesicular Body Protein 2a (CHMP2A), Ras-related protein Rab-11B (RAB11B), CD63 Antigen and CD81 Antigen are the most acknowledged (Fig. 1). Few data are currently available about the lipid composition of these vesicles. They seem to be enriched in cholesterol, sphingomyelin, ceramide and phosphatidylserine. Recent studies demonstrate that the membrane of the internal vesicles of the endosomal multivesicular bodies, which are the precursors of exosomes, are enriched in cholesterol [10]. It is interesting to note that several membrane transporters which might be also present in exosomal membranes show sensitivity to cholesterol [11,12]. Interestingly, it is also known that the exosomal membrane contains detergent-resistant subdomains similar to the lipid rafts of the plasma membrane [13]. More recent data shows a link between endocytosis of lipid rafts and its eventual secretion into exosomes [14]. One of the most interesting roles linked to the presence of mRNA or miRNA into exosomes (Fig. 1), is the transfer of nucleic acids to target cells and affect gene expression [8]. The importance of the identification of exosomal macromolecular components derives from the crucial role that they may play in many processes directly or indirectly connected with cellular functions such as inflammation, immune response, angiogenesis, cell death, neurodegenerative diseases and cancer [15]. Since exosomes, released by the different tissues, can be collected from body fluids, their cargos represent possible disease markers and, hence, therapeutic tools. For instance, understanding the exosomal cargo modifications during inflammatory processes, will provide valuable insights into the possibility of predicting and/or monitoring pathological processes before they manifest or during their progress in humans and animals. (See Table 1.)

2. Inflammation

Inflammation has the physiological purpose of restoring tissue homeostasis. However, uncontrolled or unresolved inflammation can lead to tissue damage causing several diseases characterized by chronic inflammatory states [16]. Indeed, pathways of systemic inflammation have been recognized as essential components in the pathogenesis of several diseases such as cancer, inflammatory bowel diseases, type 2 diabetes, obesity, rheumatoid arthritis and neurodegenerative diseases [17]. Among a plurality of signalling cascades, exosomes seem to play a crucial role in inflammation processes thanks to their capability to carry inflammatory modulators such as miRNA and proteins which can act on close as well as distant target tissues.

2.1. Cancer

2.1.1. Relationship between exosome inflammation and cancer

Inflammatory responses are involved in all stages of tumour development, including initiation, promotion, malignant conversion, invasion, and metastasis. Furthermore, inflammation plays a decisive role also in immune surveillance and resistance to therapy [18,19]. Indeed, in the 2011 Hanahan and Weinberg identified the inflammation as one of the hallmark capabilities which virtually all cancers must acquire [20]. A growing number of studies demonstrates that exosomes play a pivotal role to shape inflammatory microenvironment of tumours. For instance, exosomes derived from gastric cancer cells, stimulate the activation of the NF-κB pathway in macrophages, leading to increased levels of pro-inflammatory factors, which in turn promote tumour cell proliferation and migration [21]. The NF-κB signalling pathway is active in a large number of tumours among which breast cancer. Interestingly, a novel mechanism for the activation of this pathway involves palmitoylated proteins which are present on the surface of exosomes secreted by breast cancer cells. Through this mechanism, circulating exosomes are used by breast cancer cells to induce pro-inflammatory activation of macrophages resulting in secretion of pro-inflammatory cytokines, such as Interleukin 6 (IL-6), Tumour Necrosis Factor alpha (TNFα), Granulocyte Colony-Stimulating Factor (GCSF), and Chemokine C-C motif Ligand 2 (CCL2) [22].

2.1.2. Exosomes in metastasis development

Exosomes also promote metastasis development. As an example, miR-21 and miR-29a which are enclosed in tumour-secreted exosomes, trigger a pro-metastatic inflammatory response mediated by Toll-like receptor [23]. Another study demonstrated that liver metastases are triggered by the exosomal macrophage Migration Inhibitory Factor (MIF). Indeed, in patients with liver metastasis exosomes from stage I Pancreatic Ductal Adeno Carcinoma (PDAC) showed a higher level of MIF than in patients without metastatic progression. Therefore, exosomal MIF may be a prognostic marker for the development of PDAC liver metastasis [24].

2.1.3. Exosomes in cancer initiation and progression

The initiation and progression of liver cancer are strictly dependent on the local microenvironment. Exosomes derived from hepatocellular carcinoma cell line HepG2 can cause significant transcriptomic alterations in adipocytes stimulating their typical inflammatory phenotype. Indeed, adipocytes play an important role in non-alcoholic fatty liver disease, which is a significant risk factor for hepatocellular carcinoma. *In vivo* experiments, showed that the exosomes are able to promote tumour growth, angiogenesis, and macrophage recruitment in mouse [25]. Exosomes facilitate tumour development also through a remodelling of the immunity based pathways. Exosomes from human prostate cancer cell line, LNCaP, inhibit T-cell proliferation and promote T-cell apoptosis through the FasL pathway [26]. The vesicles derived from head and neck squamous cell carcinoma were also shown to induce T-cells apoptosis. In particular, it was demonstrated that tumour-derived vesicles contribute to tumour escape through the demise of the antitumor CD8(+) effector [27]. In addition, exosomes from nasopharyngeal carcinoma inhibit T cell proliferation using a mechanism that consists on the one hand, in decreasing phosphorylation of ERK, STAT1 and STAT3, and on the other, in increasing phosphorylation of STAT5. The phosphorylated factors behave as transcription activators in the recipient T cells and are also involved in inflammation. These tumour-derived exosomes are enriched in miRNA(s) which downregulate the MARK1 signalling pathway, affecting T cells proliferation and differentiation [28].

2.2. Inflammatory bowel diseases

Every day numerous bacteria and many dietary-derived antigens get in touch with intestinal epithelial cells without causing immune reactions. Intestinal mucosal homeostasis relies on the ability of the local immune system not only to maintain tolerance towards normal microbiota and food proteins but also to initiate efficient immune responses towards enteric pathogens. Inflammatory bowel diseases (IBDs) are a set of chronic disorders, that arise in genetically predisposed individuals, due to failure in maintaining intestinal homeostasis [29–31]. Many mechanisms have been proposed, by which immuno-tolerance is maintained through regulation of activated T cells. One of these involves the immunosuppressive activity of exosomes. Indeed, transfer of

normal intestine exosomes into mice with IBD decreases the severity of the disorder [32]. On the contrary, transfer of exosomes from IBD patients to human colonocytes cell line DLD-1 causes an increase of the pro-inflammatory IL-8 [33]. Macrophages are also essential for the maintenance of intestinal homeostasis, and their activation has been proposed to be critical to the pathogenesis of IBD [34]. Circulating exosomes participate in intercellular communication and can mediate the immune response through the modulation of macrophage activity. In serum exosomes from mice with induced acute colitis, 56 proteins are differentially expressed with respect to control exosomes. Most of these proteins are acute-phase proteins and immunoglobulins that are mainly involved in the complement and coagulation cascade [35]. Another important mechanism for maintaining a functional intestinal barrier is the epithelial restoring following injury. A crucial player of this process is annexin-1 which significantly contributes to the process of healing the mucosal damage and is overexpressed during the inflammatory response. Annexin-1 is released as a component of extracellular exosomes derived from intestinal epithelial cells. Patients with IBD have an elevated number of such vesicles secreted in serum. This makes annexin-1-containing exosomes a potential biomarker of intestinal mucosal inflammation [36]. Another even more promising biomarker for these pathologies is represented by the Proteasome Subunit Alpha type 7 (PSMA7) which can be detected in salivary exosomes isolated from patients with IBD. These patients showed marked differences in the PSMA7 expression compared to exosome isolated from healthy subjects [37].

2.3. Kidney diseases

2.3.1. Exosomes, inflammation and kidney diseases

In kidney diseases, extracellular vesicles promote or are linked to thrombosis, inflammation and immune-mediated disease [38]. Since exosomes can be collected from urine, their cargos may be utilized as non-invasive biomarkers of these pathologies. The association between inflammation and kidney diseases is supported by the finding that mice with acute and chronic kidney injury have an increased amount of exosomes carrying the inflammatory chemokine CCL2 mRNA with respect to control mice. The increase of CCL2 in exosomes is also observed after treatment of tubular epithelial cells (TECs) with Albumin, which induces tubule-interstitial inflammation. These exosomes can be internalized by macrophages with a consequent enhanced inflammatory response and macrophage migration. Mice subjected to tail vein injection of exosomes isolated by BSA-treated TECs develop tubular injury and renal inflammation. The importance of the exosomal CCL2 in this process was demonstrated by the injection of exosomes from BSA-treated TECs which are deficient for CCL2. Mice subjected to this treatment develop less severe inflammation. Finally, in humans, the increased proteinuria of nephropathy patients correlates with an extremely high content of CCL2 mRNA in exosomes [39]. Among other cargos that significantly increase in urinary exosomes under renal injury, there is the mRNA of the activating transcriptional factor 3 (ATF3). ATF3 is an “adaptive response” gene used by the cell to attenuate injury due to harmful cellular changes, such as renal ischemia reperfusion and, indeed, plays an anti-apoptotic and anti-inflammatory role. The mRNA of ATF3 is 60-fold higher in urinary exosomes from patients with acute kidney injury than in controls [40].

2.3.2. Exosomal OCTN2 as a biomarker of inflammatory-based kidney diseases

A novel suggested biomarker of inflammatory-based kidney pathologies is a protein belonging to the SLCs superfamily, the SLC22A5 also known as OCTN2, an acronym standing for Organic Cation Transporter Novel 2. OCTN2 is a sodium-dependent carnitine transporter which has the role of mediating the accumulation of carnitine into cells of many tissues. This process is critical for maintaining the homeostasis of carnitine that is an essential cofactor for fatty acid β-oxidation. This pathway occurs in mitochondria where carnitine allows the transport of fatty acyl moieties into the matrix as acylcarnitines, using a shuttle system composed by Carnitine Palmitoyltransferase 1 (CPT1), Carnitine/Acylcarnitine Carrier (CAC) and Carnitine Palmitoyltransferase 2 (CPT2) (Fig. 2) [41]. Peroxisomes can also perform β-oxidation but the mechanisms and the role of carnitine in the transport of fatty acyl groups through the peroxisomal membrane are less known [42]. Despite its importance, the endogenous synthesis of carnitine is not sufficient to guarantee cell homeostasis in human. More than 50% of the required carnitine is furnished by diet. Together with the ingested carnitine, the fraction reabsorbed at the kidney level, is critical for maintaining the homeostasis. This fraction is also crucial for compensating the insufficient ingestion of carnitine, for example in the case of vegetarian subjects. In these cases, renal reabsorption of carnitine increases through the augmented expression of OCTN2 [43]. A similar phenomenon also occurs during exercise which is a recognized physiological modulator of lipid metabolism [44]. The demonstration of the major role of OCTN2 in the above mentioned metabolism, is given by the association of inherited OCTN2 defects with a human pathology known as primary carnitine deficiency which is characterized by muscle weakness, encephalopathy, cardiomyopathy, confusion, vomiting and hypoglycaemia. Several inflammatory-related diseases, such as diabetes mellitus, chronic renal failure, cardiomyopathy, cirrhosis and sepsis show carnitine homeostasis derangement [45,46] in which OCTN2 may be involved. According to the relationships of carnitine with the inflammatory states, administration of this cofactor to a patient in haemodialysis significantly reduces serum C-Reactive Protein (CRP) and Serum Amyloid A (SAA), two systemic inflammation markers [47]. Recent studies suggest a more direct association between OCTN2 and inflammation. OCTN2 polymorphisms are indeed causative of Inflammatory Bowel Disease and in particular of Crohn's disease [47]. However, the exact mechanisms underlying this relationship is still not clearly understood. In this frame, the positive effect of cytokines on the OCTN2 expression has been described [48,49]. The interest in studying exosomal OCTN2 derives from its linkage with inflammation and from its crucial localization in kidney, where the transporter significantly contributes to the carnitine homeostasis [50]. It has been demonstrated that exosomal OCTN2 derived from HEK293 cell media and human urine shows all the main features of the cellular OCTN2 such as sodium and pH dependence of the carnitine uptake. Furthermore, the carnitine uptake is inhibited by acetyl carnitine but not by amino acids or NEM as previously reported [51]. The apparent molecular mass of exosomal OCTN2 is about 70 kDa which is much higher than the theoretical mass of the transporter demonstrating that it is glycosylated as in the plasma membrane [51]. This finding suggests that it is correctly folded. Interestingly, the transporter level in HEK 293 exosomes increases as a consequence of cell treatment with cytokine INF-γ indicating that the inflammatory signal is exported from cells *via* exosomal OCTN2, among other molecular mechanisms. According to the increased expression induced by INF-γ, the sodium-dependent carnitine transport mediated by the exosomal OCTN2 increases, as well. Even though additional studies are required for understanding the role of OCTN2 in the cell to cell signalling, exosomes very probably represent a mechanism for transferring functional OCTN2 to the inflammation site for recovering the carnitine transport capacity of altered renal cells. This, in turn, could represent a benefit for controlling the inflammatory state [51].

2.4. Obesity and diabetes

Adipose tissue is considered a ‘master regulator’ of systemic energy homeostasis by its involvement in the regulation of the other metabolic organs, including liver, pancreas, kidney and skeletal muscle. Thus, dysfunction of the adipose tissue is associated with obesity-related comorbidities, such as cardiovascular disease, hypertension, dyslipidemia, insulin resistance, and diabetes mellitus. In this scenario, exosomes represent a putative mechanism by which adipocytes can influence

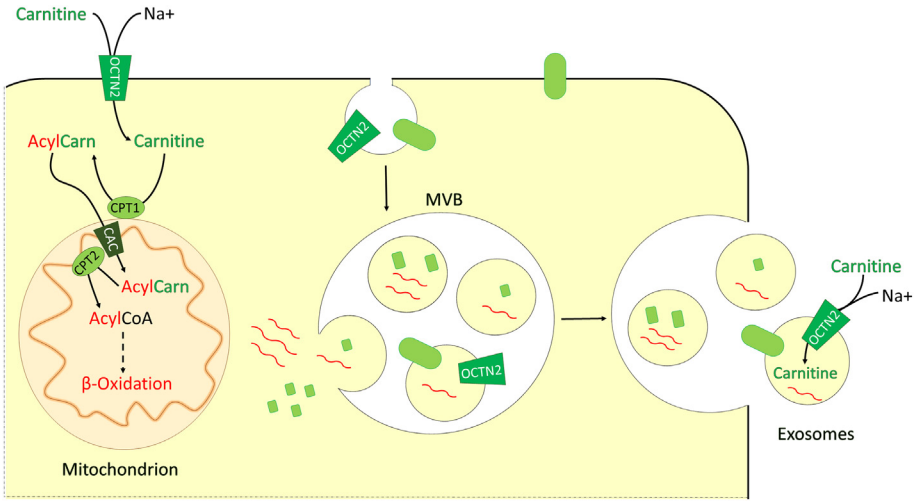

Fig. 2. Role of OCTN2 in carnitine homeostasis. The sodium-dependent carnitine uptake is mediated by OCTN2. Then carnitine allows fatty acids to enter into mitochondrial matrix where they will be subjected to β-oxidation. Carnitine Palmitoyltransferase 1 (CPT1), Carnitine/Acylcarnitine Carrier (CAC) and Carnitine Palmitoyltransferase 2 (CPT2).

other organ systems [52]. According to this hypothesis, it was found that exosomes isolated from adipose tissue from obese subjects contain an altered level of 55 miRNAs which mainly regulate TGF-β and Wnt/β-catenin signalling. The interaction between Wnt/β-catenin and TGF-β signalling is important in the development and progression of chronic inflammation which characterizes the obesity condition [53]. Exosomes like vesicles released by adipose tissue of obese mice are taken up by mononuclear cells inducing their differentiation into active macrophages with an increased release of TNF-α and IL-6. This exosome-mediated activation involves the TLR4/TRIF pathway and may also be a factor involved in obesity-associated insulin resistance [54]. The relationship between exosomes, obesity and insulin resistance was recently confirmed. Adipose tissue macrophages in obese mice secrete exosomes containing miRNAs which are able to induce glucose intolerance and insulin resistance in lean mice [55]. On the contrary, exosomes from Adipose-Derived Stem Cells (ADSC) drive the improvement of insulin sensitivity and the reduction of inflammation-inducing anti-inflammatory M2 phenotypes. This suggests an exosome-mediated cross-talk between ADSC and macrophages that facilitates immune and metabolic homeostasis in white adipose tissue [56].

2.5. Rheumatoid arthritis

Rheumatoid Arthritis (RA) is a chronic, inflammatory and systemic autoimmune disease caused by inflammation of the synovium. It leads to a long-term joint damage, resulting in chronic pain, loss of function and even disability. The pathogenic mechanism of RA is not fully understood.

However recent studies demonstrate the involvement of extracellular vesicles. The role of exosomes in the aetiology of this pathology is supposed by the observation that vesicles derived from synovial fibroblasts treated with Interleukin-1β induce osteoarthritis-like changes in *in vitro* and in *ex vivo* models [57]. Exosomes produced by synovial fibroblasts obtained from individuals with RA contain a membrane-bound form of TNF-α. These exosomes can enter into anti-CD3-activated T cells causing AKT and NF-kB activation. Moreover, after uptake of exosomes, the activated T cells become resistant to apoptosis [58]. Another observation that supports the role of exosomes in the RA aetiology is that synovial exosomes isolated from a patient with this disorder show the presence of citrullinate proteins. This post translational modification occurs through the action of a calcium dependent peptidyl arginine deiminase which catalyses the conversion of arginine into citrulline. The importance of this finding, in the Rheumatoid Arthritis scenario, is related to the fact that citrullination represents a key step in the transformation of non-immunogenic proteins to auto-immunogenic ones [59]. Exosomes can also exert an anti-inflammatory action.

Indeed, exosomes derived from mesenchymal stem cells protect cartilage from degradation and promote its repair in osteoarthritis [60,61].

2.6. Neuroinflammation

Neuroinflammation is a common feature of a large number of neurodegenerative diseases such as Alzheimer's Disease (AD), Parkinson's Disease (PD) and Amyotrophic Lateral Sclerosis (ALS). Indeed all these disorders are characterized by glial activation and high level of pro-inflammatory cytokine production in the central nervous system (CNS) [62]. Recent studies highlight that exosomes are involved in the pathogenesis of neuroinflammatory disorders [63]. Proteins such as α-synuclein, amyloid β and prions which are involved in diseases such as PD, AD and Creutzfeldt-Jacob, are moved from one cell to another into exosomes triggering the inflammatory cascade [63]. Besides this, stimulation of peripheral immune response is often associated with inflammation in the central nervous system, suggesting a cross talk between central and peripheral immune systems. Indeed, experimental evidence indicate that purified serum-derived exosomes from LPS-treated mice, can induce both systemic and CNS inflammation, even though the mechanism by which this happens is not well understood [62]. Moreover, inhibiting the excessive inflammatory response after traumatic brain injury, improves the neurologic outcome. It was recently found that after brain injury, the level of miR-124-3p increases. This miRNA is able to reduce inflammation of neurons and promote neurite outgrowth after scratch injury. Given the relevance of exosomes in brain physiopathology, they are considered a novel therapeutic tool for delivering anti-inflammatory mediators to the CNS [25].

3. Conclusions

A growing number of evidence indicates that exosomes can modulate gene expression and cell function, playing a crucial role in several processes such as inflammation and immune response. On the basis of the results so far provided, it is clear that the exosomal components change in relationship to the physio-pathological state of the cells from which they are released. Hence, exosomal components represent promising candidates for novel non-invasive biomarkers, since these subtype of vesicles can be collected from body fluids such as blood, urine and saliva. The methods currently employed for isolation are four: i) The ultracentrifugation is often used in combination with the sucrose density gradients.

In this procedure, sequential centrifugations at increasing speeds are performed. The method requires a large amount of starting material with relatively low yields of good quality exosomes. ii) The size-based

isolation approach includes the ultrafiltration procedure and HPLC (High-Performance Liquid Chromatography)-based protocols. Both protocols allow obtaining exosome-enriched samples. iii) The exosomes precipitation procedure is a time-saving technique, which uses volume-excluding polymers such as polyethylene glycols to collect exosomes as a pellet at the end of a low-speed centrifugation. iv) The affinity-based isolation approach employs antibodies against exosomal markers such as CD63, CD9 and Alix which are immobilized on magnetic beads, chromatography matrices, or microfluidic devices. The above-described strategies are a good starting point to isolate exosomes for detection and screening of human pathologies. However, additional efforts are needed, not only to further investigate the relationship between the variation of exosomal components and the disease progression, but also to improve the methodologies for exosome isolation, to ensure reliable and reproducible results.

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References

[1] E. Chargaff, R. West, The biological significance of the thromboplastic protein of blood, J. Biol. Chem. 166 (1) (1946) 189–197.
[2] P. Wolf, The nature and significance of platelet products in human plasma, Br. J. Haematol. 13 (3) (1967) 269–288.
[3] R.M. Johnstone, M. Adam, J.R. Hammond, L. Orr, C. Turbide, Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes), J. Biol. Chem. 262 (19) (1987) 9412–9420.
[4] M. Colombo, C. Moita, G. van Niel, J. Kowal, J. Vigneron, P. Benaroch, N. Manel, L.F. Moita, C. Thery, G. Raposo, Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles, J. Cell Sci. 126 (2013) 5553–5565 Pt 24.
[5] S. Stuffers, C. Sem Wegner, H. Stenmark, A. Brech, Multivesicular endosome biogenesis in the absence of ESCRTs, Traffic 10 (7) (2009) 925–937.
[6] B. Gyorgy, T.G. Szabo, M. Pasztoi, Z. Pal, P. Misjak, B. Aradi, V. Laszlo, E. Pallinger, E. Pap, A. Kittel, G. Nagy, A. Falus, E.I. Buzas, Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles, Cell. Mol. Life Sci. 68 (16) (2011) 2667–2688.
[7] C. Thery, M. Ostrowski, E. Segura, Membrane vesicles as conveyors of immune responses, Nat. Rev. Immunol. 9 (8) (2009) 581–593.
[8] H. Valadi, K. Ekstrom, A. Bossios, M. Sjostrand, J.J. Lee, J.O. Lotvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (6) (2007) 654–659.
[9] G. Raposo, W. Stoorvogel, Extracellular vesicles: exosomes, microvesicles, and friends, J. Cell Biol. 200 (4) (2013) 373–383.
[10] W. Mobius, E. van Donselaar, Y. Ohno-Iwashita, Y. Shimada, H.F. Heijnen, J.W. Slot, H.J. Geuze, Recycling compartments and the internal vesicles of multivesicular bodies harbor most of the cholesterol found in the endocytic pathway, Traffic 4 (4) (2003) 222–231.
[11] D. Dickens, G.N. Chiduza, G.S. Wright, M. Pirmohamed, S.V. Antonyuk, S.S. Hasnain, Modulation of LAT1 (SLC7A5) transporter activity and stability by membrane cholesterol, Sci. Rep. 7 (2017) 43580.
[12] S. Keerthikumar, D. Chisanga, D. Ariyaratne, H. Al Saffar, S. Anand, K. Zhao, M. Samuel, M. Pathan, M. Jois, N. Chilamkurti, L. Gangoda, S. Mathivanan, ExoCarta: a web-based compendium of exosomal cargo, J. Mol. Biol. 428 (4) (2016) 688–692.
[13] M. Colombo, G. Raposo, C. Thery, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014) 255–289.
[14] S.S. Tan, Y. Yin, T. Lee, R.C. Lai, R.W. Yeo, B. Zhang, A. Choo, S.K. Lim, Therapeutic MSC exosomes are derived from lipid raft microdomains in the plasma membrane, J. Extracell. Vesicles 2 (2013).
[15] J. Howitt, A.F. Hill, Exosomes in the Pathology of Neurodegenerative Diseases, J. Biol. Chem. 291 (52) (2016) 26589–26597.
[16] M.A. Sugimoto, L.P. Sousa, V. Pinho, M. Perretti, M.M. Teixeira, Resolution of inflammation: what controls its onset? Front. Immunol. 7 (2016) 160.
[17] R. Scrivo, M. Vasile, I. Bartosiewicz, G. Valesini, Inflammation as “common soil” of the multifactorial diseases, Autoimmun. Rev. 10 (7) (2011) 369–374.
[18] S.I. Grivennikov, F.R. Greten, M. Karin, Immunity, inflammation, and cancer, Cell 140 (6) (2010) 883–899.
[19] F. Colotta, P. Allavena, A. Sica, C. Garlanda, A. Mantovani, Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability, Carcinogenesis 30 (7) (2009) 1073–1081.
[20] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674.
[21] L. Wu, X. Zhang, B. Zhang, H. Shi, X. Yuan, Y. Sun, Z. Pan, H. Qian, W. Xu, Exosomes derived from gastric cancer cells activate NF-kappaB pathway in macrophages to promote cancer progression, Tumour Biol. 37 (9) (2016) 12169–12180.
[22] A. Chow, W. Zhou, L. Liu, M.Y. Fong, J. Champer, D. Van Haute, A.R. Chin, X. Ren, B.G. Gugiu, Z. Meng, W. Huang, V. Ngo, M. Kortylewski, S.E. Wang, Macrophage immunomodulation by breast cancer-derived exosomes requires Toll-like receptor 2-mediated activation of NF-kappaB, Sci. Rep. 4 (2014) 5750.
[23] M. Fabbri, A. Paone, F. Calore, R. Galli, E. Gaudio, R. Santhanam, F. Lovat, P. Fadda, C. Mao, G.J. Nuovo, N. Zanesi, M. Crawford, G.H. Ozer, D. Wernicke, H. Alder, M.A. Caligiuri, P. Nana-Sinkam, D. Perrotti, C.M. Croce, MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response, Proc. Natl. Acad. Sci. U. S. A. 109 (31) (2012) E2110–E2116.
[24] B. Costa-Silva, N.M. Aiello, A.J. Ocean, S. Singh, H. Zhang, B.K. Thakur, A. Becker, A. Hoshino, M.T. Mark, H. Molina, J. Xiang, T. Zhang, T.M. Theilen, G. Garcia-Santos, C. Williams, Y. Ararso, Y. Huang, G. Rodrigues, T.L. Shen, K.J. Labori, I.M. Lothe, E.H. Kure, J. Hernandez, A. Doussot, S.H. Ebbesen, P.M. Grandgenett, M.A. Hollingsworth, M. Jain, K. Mallya, S.K. Batra, W.R. Jarnagin, R.E. Schwartz, I. Matei, H. Peinado, B.Z. Stanger, J. Bromberg, D. Lyden, Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver, Nat. Cell Biol. 17 (6) (2015) 816–826.
[25] S. Huang, X. Ge, J. Yu, Z. Han, Z. Yin, Y. Li, F. Chen, H. Wang, J. Zhang, P. Lei, Increased miR-124-3p in microglial exosomes following traumatic brain injury inhibits neuronal inflammation and contributes to neurite outgrowth via their transfer into neurons, FASEB J. 32 (1) (2018) 512–528.
[26] A.J. Abusamra, Z. Zhong, X. Zheng, M. Li, T.E. Ichim, J.L. Chin, W.P. Min, Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis, Blood Cells Mol. Dis. 35 (2) (2005) 169–173.
[27] E.U. Wieckowski, C. Visus, M. Szajnik, M.J. Szczepanski, W.J. Storkus, T.L. Whiteside, Tumor-derived microvesicles promote regulatory T cell expansion and induce apoptosis in tumor-reactive activated CD8+ T lymphocytes, J. Immunol. 183 (6) (2009) 3720–3730.
[28] S.B. Ye, Z.L. Li, D.H. Luo, B.J. Huang, Y.S. Chen, X.S. Zhang, J. Cui, Y.X. Zeng, J. Li, Tumor-derived exosomes promote tumor progression and T-cell dysfunction through the regulation of enriched exosomal microRNAs in human nasopharyngeal carcinoma, Oncotarget 5 (14) (2014) 5439–5452.
[29] J. Dupaul-Chicoine, M. Dagenais, M. Saleh, Crosstalk between the intestinal microbiota and the innate immune system in intestinal homeostasis and inflammatory bowel disease, Inflamm. Bowel Dis. 19 (10) (2013) 2227–2237.
[30] A.T. Xu, J.T. Lu, Z.H. Ran, Q. Zheng, Exosome in intestinal mucosal immunity, J. Gastroenterol. Hepatol. 31 (10) (2016) 1694–1699.
[31] L. Jiang, Y. Shen, D. Guo, D. Yang, J. Liu, X. Fei, Y. Yang, B. Zhang, Z. Lin, F. Yang, X. Wang, K. Wang, J. Wang, Z. Cai, Corrigendum: EpCAM-dependent extracellular vesicles from intestinal epithelial cells maintain intestinal tract immune balance, Nat. Commun. 8 (2017) 16006.
[32] L. Jiang, Y. Shen, D. Guo, D. Yang, J. Liu, X. Fei, Y. Yang, B. Zhang, Z. Lin, F. Yang, X. Wang, K. Wang, J. Wang, Z. Cai, EpCAM-dependent extracellular vesicles from intestinal epithelial cells maintain intestinal tract immune balance, Nat. Commun. 7 (2016) 13045.
[33] S. Mitsuhashi, L. Feldbrugge, E. Csizmadia, M. Mitsuhashi, S.C. Robson, A.C. Moss, Luminal extracellular vesicles (EVs) in inflammatory bowel disease (IBD) exhibit proinflammatory effects on epithelial cells and macrophages, Inflamm. Bowel Dis. 22 (7) (2016) 1587–1595.
[34] E.C. Steinbach, S.E. Plevy, The role of macrophages and dendritic cells in the initiation of inflammation in IBD, Inflamm. Bowel Dis. 20 (1) (2014) 166–175.
[35] W.Y. Wong, M.M. Lee, B.D. Chan, R.K. Kam, G. Zhang, A.P. Lu, W.C. Tai, Proteomic profiling of dextran sulfate sodium induced acute ulcerative colitis mice serum exosomes and their immunomodulatory impact on macrophages, Proteomics 16 (7) (2016) 1131–1145.
[36] G. Leoni, P.A. Neumann, N. Kamaly, M. Quiros, H. Nishio, H.R. Jones, R. Sumagin, R.S. Hilgarth, A. Alam, G. Fredman, I. Argyris, E. Rijcken, D. Kusters, C. Reutelingsperger, M. Perretti, C.A. Parkos, O.C. Farokhzad, A.S. Neish, A. Nusrat, Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair, J. Clin. Invest. 125 (3) (2015) 1215–1227.
[37] X. Zheng, F. Chen, Q. Zhang, Y. Liu, P. You, S. Sun, J. Lin, N. Chen, Salivary exosomal PSMA7: a promising biomarker of inflammatory bowel disease, Protein Cell 8 (9) (2017) 686–695.
[38] A.L. Stahl, K. Johansson, M. Mossberg, R. Kahn and D. Karpman, Exosomes and microvesicles in normal physiology, pathophysiology, and renal diseases, Pediatr. Nephrol. in press
[39] L.L. Lv, Y. Feng, Y. Wen, W.J. Wu, H.F. Ni, Z.L. Li, L.T. Zhou, B. Wang, J.D. Zhang, S.D. Crowley, B.C. Liu, Exosomal CCL2 from tubular epithelial cells is critical for albumin-induced tubulointerstitial inflammation, J. Am. Soc. Nephrol. 29 (3) (2018) 919–935.
[40] H.H. Chen, P.F. Lai, Y.F. Lan, C.F. Cheng, W.B. Zhong, Y.F. Lin, T.W. Chen, H. Lin, Exosomal ATF3 RNA attenuates pro-inflammatory gene MCP-1 transcription in renal ischemia-reperfusion, J. Cell. Physiol. 229 (9) (2014) 1202–1211.
[41] L. Console, N. Giangregorio, C. Indiveri, A. Tonazzi, Carnitine/acylcarnitine translocase and carnitine palmitoyltransferase 2 form a complex in the inner mitochondrial membrane, Mol. Cell. Biochem. 394 (1–2) (2014) 307–314.
[42] R.J. Wanders, H.R. Waterham, S. Ferdinandusse, Metabolic interplay between peroxisomes and other subcellular organelles including mitochondria and the endoplasmic reticulum, Front. Cell Develop. Biol. 3 (2015) 83.
[43] F.B. Stephens, K. Marimuthu, Y. Cheng, N. Patel, D. Constantin, E.J. Simpson, P.L. Greenhaff, Vegetarians have a reduced skeletal muscle carnitine transport capacity, Am. J. Clin. Nutr. 94 (3) (2011) 938–944.

[44] M. Hoene, J. Li, Y. Li, H. Runge, X. Zhao, H.U. Haring, R. Lehmann, G. Xu, C. Weigert, Muscle and liver-specific alterations in lipid and acylcarnitine metabolism after a single bout of exercise in mice, Sci. Rep. 6 (2016) 22218.
[45] D. Mamoulakis, E. Galanakis, E. Dionyssopoulou, A. Evangeliou, S. Sbyrakis, Carnitine deficiency in children and adolescents with type 1 diabetes, J. Diabetes Complicat. 18 (5) (2004) 271–274.
[46] J.L. Flanagan, P.A. Simmons, J. Vehige, M.D. Willcox, Q. Garrett, Role of carnitine in disease, Nutr. Metab. 7 (2010) 30.
[47] S. Khalatbari-Soltani, H. Tabibi, Inflammation and L-carnitine therapy in hemodialysis patients: a review, Clin. Exp. Nephrol. 19 (3) (2015) 331–335.
[48] M. Fujiya, Y. Inaba, M.W. Musch, S. Hu, Y. Kohgo, E.B. Chang, Cytokine regulation of OCTN2 expression and activity in small and large intestine, Inflamm. Bowel Dis. 17 (4) (2011) 907–916.
[49] F. Ingoglia, R. Visigalli, B.M. Rotoli, A. Barilli, B. Riccardi, P. Puccini, M. Milioli, M. Di Lascia, G. Bernuzzi, V. Dall'Asta, Human macrophage differentiation induces OCTN2-mediated L-carnitine transport through stimulation of mTOR-STAT3 axis, J. Leukoc. Biol. 101 (3) (2017) 665–674.
[50] L. Pochini, M. Scalise, M. Galluccio, C. Indiveri, OCTN cation transporters in health and disease: role as drug targets and assay development, J. Biomol. Screen. 18 (8) (2013) 851–867.
[51] L. Console, M. Scalise, A. Tonazzi, N. Giangregorio, C. Indiveri, Characterization of exosomal SLC22A5 (OCTN2) carnitine transporter, Sci. Rep. 8 (1) (2018) 3758.
[52] F. Pardo, R. Villalobos-Labra, B. Sobrevia, F. Toledo, L. Sobrevia, Extracellular vesicles in obesity and diabetes mellitus, Mol. Asp. Med. 60 (2018) 81–91.
[53] S.C. Ferrante, E.P. Nadler, D.K. Pillai, M.J. Hubal, Z. Wang, J.M. Wang, H. Gordish-Dressman, E. Koeck, S. Sevilla, A.A. Wiles, R.J. Freishtat, Adipocyte-derived exosomal miRNAs: a novel mechanism for obesity-related disease, Pediatr. Res. 77 (3) (2015) 447–454.
[54] Z.B. Deng, A. Poliakov, R.W. Hardy, R. Clements, C. Liu, Y. Liu, J. Wang, X. Xiang, S. Zhang, X. Zhuang, S.V. Shah, D. Sun, S. Michalek, W.E. Grizzle, T. Garvey, J. Mobley, H.G. Zhang, Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance, Diabetes 58 (11) (2009) 2498–2505.
[55] W. Ying, M. Riopel, G. Bandyopadhyay, Y. Dong, A. Birmingham, J.B. Seo, J.M. Ofrecio, J. Wollam, A. Hernandez-Carretero, W. Fu, P. Li, J.M. Olefsky, Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity, Cell 171 (2) (2017) 372-384 e12.
[56] H. Zhao, Q. Shang, Z. Pan, Y. Bai, Z. Li, H. Zhang, Q. Zhang, C. Guo, L. Zhang, Q. Wang, Exosomes from adipose-derived stem cells attenuate adipose inflammation and obesity through polarizing M2 macrophages and beiging in white adipose tissue, Diabetes 67 (2) (2018) 235–247.
[57] T. Kato, S. Miyaki, H. Ishitobi, Y. Nakamura, T. Nakasa, M.K. Lotz, M. Ochi, Exosomes from IL-1beta stimulated synovial fibroblasts induce osteoarthritic changes in articular chondrocytes, Arthritis Res. Ther. 16 (4) (2014) R163.
[58] H.G. Zhang, C. Liu, K. Su, S. Yu, L. Zhang, S. Zhang, J. Wang, X. Cao, W. Grizzle, R.P. Kimberly, A membrane form of TNF-alpha presented by exosomes delays T cell activation-induced cell death, J. Immunol. 176 (12) (2006) 7385–7393.
[59] K. Skriner, K. Adolph, P.R. Jungblut, G.R. Burmester, Association of citrullinated proteins with synovial exosomes, Arthritis Rheum. 54 (12) (2006) 3809–3814.
[60] S. Zhang, W.C. Chu, R.C. Lai, S.K. Lim, J.H. Hui, W.S. Toh, Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration, Osteoarthr. Cartil. 24 (12) (2016) 2135–2140.
[61] S. Cosenza, M. Ruiz, K. Toupet, C. Jorgensen, D. Noel, Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis, Sci. Rep. 7 (1) (2017) 16214.
[62] J.J. Li, B. Wang, M.C. Kodali, C. Chen, E. Kim, B.J. Patters, L. Lan, S. Kumar, X. Wang, J. Yue, F.F. Liao, In vivo evidence for the contribution of peripheral circulating inflammatory exosomes to neuroinflammation, J. Neuroinflammation 15 (1) (2018) 8.
[63] A. Gupta, L. Pulliam, Exosomes as mediators of neuroinflammation, J. Neuroinflammation 11 (2014) 68.