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Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds

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Abstract

A rapidly growing body of experimental evidence has begun to shed light on the wide ranging molecular mechanisms which modulate intra- and inter-cellular communications. A substantial quantity of the available knowledge has only been uncovered in recent years, and we are learning that donor cells release nanovesicles, known as exosomes, which regulate the cellular behavior of recipient cells following uptake. Based on the impressive capacity of exosomes in delivering their "payload", different therapeutic agents, are currently being tested using this delivery method for more effective therapy. This review summarizes the most recent developments in exosome bioactivities and discusses the biochemical nature of exosomes and their biogenesis. It also summarizes the use of exosomes as delivery vehicles for drugs and natural compounds to the targeted site.

Keywords: Exosomes, Drug delivery, Phytochemicals, Curcumin.

1. Introduction

Cells release a range of extracellular vesicles (EVs), either as a reaction to specific stimuli, or as part of normal physiological conditions. These are derived from the endosomal pathway or the plasma membrane, and can be categorized according to their dimensions and cellular origin. Apoptotic bodies are fragments left by dying cells and have a diameter ranging from 1,000 to 5,000 nm. Microvesicles, also known as ectosomes, originate from budding cellular membranes and have a size of 200-1,000 nm. Exosomes are the smallest EVs, with a diameter of 30-150 nm, and have an endosomal origin (Marote et al., 2016; McGough and Vincent, 2016; Whiteside, 2016). What's common among EVs is their role as carriers of molecular information as part of cell-to-cell communication, transferring cargo molecules involved in both physiological and pathological processes from parent to recipient cells (Bang and Thum, 2012; Kalluri, 2016; Schorey and Bhatnagar, 2008). This intercellular trafficking can be addressed to neighboring cells, as a form of paracrine signaling, and/or to distant cells, acting as a type of endocrine signaling. This is possible due to the presence of a specific molecule signature on their surfaces which makes them capable of targeting recipient cells in a very particular way. In addition, the ubiquitous presence of exosomes in all body fluids reveals their stability in extracellular environments and also explains their potential for endocrine communication. Clinical interest in these vesicles has recently emerged, due to their potential use in diagnostic applications and as part of new therapeutic strategies. Exosomes represent ideal biomarkers, in fact, aside from their presence in all body fluids, which makes their analysis cheap and minimally invasive, they carry information specific to their progenitor cells fulfilling the prerequisite of specificity (Stremersch et al., 2016).

Considerable attention has recently been focused on natural compounds with health benefits (Marchese et al., 2016a; Marchese et al., 2016b; Nabavi et al., 2015; Russo et al., 2016). A variety of clinical evidence has shown that natural compounds can possess beneficial effects impacting a number of human diseases which include neurodegenerative and cardiovascular diseases, as well as

numerous forms of cancer (Estruch et al., 2013; Orhan et al., 2015; Pazoki-Toroudi et al., 2016; Tangney and Rasmussen, 2013). In addition, several epidemiological studies have also shown that a high consumption of fruit and vegetables, which contain high concentrations of naturally bioactive compounds, can prevent a number of non-communicable diseases such as cancer, Alzheimer's disease, etc. (Dai et al., 2006; Loef and Walach, 2012; Rodriguez-Casado, 2016; Zuniga et al., 2016). This phenomenon led to an upsurge in scientific studies aimed at finding novel bioactive natural compounds with high efficacy and low side effects. This review aims to summarize the available evidence on the biofunctional roles of exosomes and their potential use as delivery systems for natural compounds.

2. Exosome biogenesis

Exosomes are generated from late endosomes which subsequently form multivesicular bodies (MVBs) through a number of different pathways. The most characterized pathway that relies on endosomal-sorting complexes required for transport (ESCRT), which recognizes ubiquitylated proteins, while others may involve sphingomyelinases (Trajkovic et al., 2008), sphingosine-1-phosphate, and tetraspanin-enriched domains (Brinton et al., 2015). There are four ESCRT complexes, numbered from 0 to 3, which are composed of many proteins able to recognize ubiquitinated cargoes. The subunits of the ESCRT-0 complex recruit proteins for internalization, such as ubiquitinated proteins and clathrin. ESCRT -1 and 2 determine the beginning of the budding process and promote the enzymatic de-ubiquitination of cargo proteins before the formation of intraluminal vesicles (ILVs), which group to form larger membranous vesicles called MVBs in the intracellular compartment. The ESCRT-3 complex drives the final stage of membrane invagination and separation (Ha et al., 2016; McGough and Vincent, 2016). Depletion of ESCRT complexes has been found to cause a modulation in the formation of MVBs but not their complete absence, suggesting the presence of independent mechanisms. In fact, it has been demonstrated that both

ceramide-rich lipid domains and tetraspanin CD63 on the extracellular side of the membrane, lead to ILV formation (Colombo et al., 2013). MVBs may undergo different processes in the cell, and can merge either with lysosomes, which degrades their contents, or with the plasma membrane, which releases exosomes from the cell. This mechanism involves many proteins such as clathrin, coat protein complex I and II (COPI and II), soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), and GTPases (Cai et al., 2007). RNA interference-mediated knockdown of RAB27, SLP4 and SLAC2B has been shown to negatively influence exosome release, showing that these proteins play crucial roles in the secretion of exosomes (Ostrowski et al., 2010).

3. Exosome composition and transfer

The composition of the exosome bilayer reflects the molecules present in the membrane of its parent cells and allows for discrimination between exosomes derived from different cells, whether these are normal or pathological (Théry et al., 2009). Of the proteins commonly present on their surface, the most abundant are adhesion molecules belonging to the tetraspanin and integrin families as shown in Figure 1. The tetraspanin family are membrane-crossing proteins and usually associate with one another to form tetraspanin membrane domain, or with neighboring proteins like integrins. Specific tetraspanins, such as CD9, CD63, CD81 and CD82, regulate the mechanisms of fusion, migration and adhesion to target cells. The expression patterns of these tetraspanins/integrins regulate a variety of biological functions acting as adhesion molecules, including migration, adhesion and proliferation, as well as mediating the interaction between exosomes and recipient cells. Moreover, the major histocompatibility complex II (MHC-II) may be present, promoting certain T-cell specific responses (Batrakova and Kim, 2015). Tumor derived exosomes (TDEs) have a different membrane composition to exosomes derived from normal cells. In fact, prostate cancer cells have been shown to release vesicles characterized by the presence of

$\alpha_v\beta_6$ integrin, which is horizontally transferred to target cells, promoting cell-migration and metastasis (Fedele et al., 2015). Other integrins have been associated with exosome-mediated metastatic processes, such as $\alpha_6\beta_4$ and $\alpha_6\beta_1$ for lung, and $\alpha_v\beta_5$ for liver metastasis (Hoshino et al., 2015). Other protein molecules have been found bound to lipid fractions on exosomes, and are related to transport and fusion functions, such as annexins, flotillin, GTPases, RABs and ADP ribosylation factors (ARFs) (Colombo et al., 2014). The lipid composition of exosomal membranes depends on the parent cell plasma membrane type, phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols, sphingomyelins, lysobis-phosphatidic acid (bis-monoacylglycerol phosphate), phosphatidic acid, lysophosphatidylcholines, cholesterol, ceramide and phosphoglicerides have been found in these membranes (Subra et al., 2007). The intraluminal composition of the exosomal membrane depends on the parent cells, and on their cytoplasmatic content in particular. Heat shock proteins (i.e. Hsp90 and Hsp70), cytoskeletal proteins (i.e. actin, tubulin, cofilin), lipids and enzymes, along with RNAs, such as miRNAs (microRNAs), mRNAs, and other non-coding RNAs (ncRNAs) have been found in the exosomal lumen, and mitochondrial DNA (mtDNA) and single strand DNA (ssDNA) have been detected more recently (Guescini et al., 2010). When exosomes fuse with recipient cells, they release their content into the cytoplasmatic space, allowing for the horizontal transfer of their cargo. The uptake of the exosomes by the receptor cells is a very energy-dependent active process. Basically, it is receptor-mediated endocytosis in which exosomal membrane proteins and lipids, such as tetraspanins and phosphatidylserine, interact with the complementary molecules found on the plasma membranes of cells and are subsequently internalized (Escrevente et al., 2011; Morelli et al., 2004). The molecular signals carried by exosomes and/or their uptake by target cells can be triggered 1) through the direct interaction of a receptor with its ligand, 2) through adhesion molecules (i.e. integrins, ICAMs) which induce fusion and endocytosis, and 3) through the phagocytosis of opsonized exosomes (Whiteside, 2016).

4. Purification of exosomes

Different methods have been developed for extracting exosomes from cell culture media or biological fluids. The most common methods involve subsequent centrifugation steps, finishing with ultracentrifugation at 100,000 x g, for a variable period (2-5 h). The disadvantages of this strategy are its length and the high viscosity of the starting samples, which negatively affect the yield. Another technique involves sucrose density gradient ultracentrifugation, which allows the exosomal fraction to be purified further. Precipitation is also used, though this method presents poor specificity. Many commercial kits have been developed to take advantage of this technique. Size exclusion chromatography provides a high yield of isolated exosomes, maintaining their function. A filtration process can also be used to obtain exosomes and is primarily applied as a preparative method before other techniques. The combination of different approaches is recommended to increase the advantages and decrease the problems associated with single techniques (Stremersch, De Smedt, 2016; Whiteside, 2016). The deterministic lateral displacement (DLD) pillar array technique has recently been used to separate exosomes, allowing for on-chip sorting in addition to quantification (Batrakova and Kim, 2015; Wunsch et al., 2016).

5. Physiological and pathological functions of exosomes

After their discovery, exosomes were thought to be vehicles responsible for removing cell debris, such as redundant intracellular organelles. However, after these vesicles were found to stimulate the immune response, their importance in intracellular communication was considered. Upon internalization, the recipient cell responds to the transferred cargo, modulating its basal functions and gene expression. Exosomes can be found in all bodily fluids, including saliva, breast milk, blood, synovial fluid, amniotic fluid, urine, sperm and follicular fluid. They reach target cells by traveling through these body fluids, fulfilling their function as carriers of both physiological and

pathological signals. Exosomes have exceptional capacity to interact with recipient cells through adhesion proteins and vector ligands (Franz et al. , 2016; Piehl et al., 2013; Qin and Xu, 2014; Santonocito et al., 2014; Simpson et al., 2008). A variety of different types of cell may release exosomes to the extracellular medium under both *in vitro* and *in vivo* conditions. The functions of these released exosomes vary, and primarily depend on the origin of the cell/tissue. For example exosomes shed by central nervous system provide a means of communication between neural cells, as well as getting rid of waste (Frühbeis et al., 2013). In addition to mediating these vital brain functions, exosomes are involved in the pathogenesis of a range of neuroinflammatory conditions (Gupta and Pulliam, 2014). The role of exosomes as modulators of the immune system has drawn great interest, and researchers have come up with several interesting findings. Adaptive immunity and suppression of inflammation can be triggered depending on the status of the immune cells involved (Théry et al., 2009). Exosomes were found to play an important role in the mediation of adaptive and innate immune responses, taking part in antigen presentation, distributing antigens in a coordinated manner with MHC-II and MHC-I molecules (Sun et al., 2013). Exosomes can confer immune suppression through several mechanisms, for example enhancing the function of regulatory T cells, inhibiting monocyte differentiation into dendritic cells (DCs), and suppressing natural killer and CD8+ activity (Clayton et al., 2007; Liu et al., 2006). Exosomes have been conversely found to activate the immune system, and this can be mediated by activation of NK cells, B cells and by survival of hematopoietic cells. Exosomes derived from human B cells have been shown to prompt an antigen-specific MHC class II response. MHC class I responses and other co-stimulatory molecules have been expressed in DCs derived exosomes (Ohno et al., 2013). Rejection of established tumors was observed by the initiation of the cytotoxic T cell response when tumor antigen was presented by DCs derived exosomes (Zitvogel et al., 1998). Tumor cells secrete 10 times more vesicles than normal cells, and it is assumed that this is the most efficient way for tumor and metastatic information to be transferred to both normal and tumor cells (Shao et al., 2016).

Zomer et al. (Zomer et al., 2015) demonstrated that the transfer of tumor derived exosome (TDE) contents to non-malignant cells triggers the activation of tumor phenotype and metastatic properties. In fact, TDEs play key roles in many tumor-associated events, such as metastasis, migration, proliferation, angiogenesis, drug resistance and immune suppression (Shao et al., 2016). These signals can be propagated by different cargo molecules, such as miRNAs, transcripts and proteins. Many authors have demonstrated that TDEs contain the signatures of specific oncogenic or tumor-suppressing miRNAs, with miR-200 and let-7 families as respective examples (Kobayashi et al., 2014).

6. Use of Exosomes as new delivery systems

6.1. Advantages of exosomes in respect to other nanoscale drug delivery systems

During recent decades, several new nanoscale drug delivery systems have been studied to increase the efficacy and circulation time of drugs, their specificity to given targets and to reduce toxicity. The most investigated novel methods are based on natural and synthetic polymers or lipids (Kotmakçı and Çetintaş, 2015). Doxorubicin has been successfully encapsulated in liposomes (DoxilR), and paclitaxel has been encapsulated in protein-based nanoparticles (AbraxaneR) (Liu et al., 2016). Even though these liposomes possess good qualities as delivery vehicles, such as incorporation of both hydrophilic and hydrophobic drugs, and membrane penetration, they still possess some inherent disadvantages in the form of lower circulating stability, rapid clearance by phagocytosis, and increased toxicity (Ha et al., 2016). PEGylation of these particles can increase their circulation time, but the downside is the decreased biodistribution of drugs in diseased tissues, due to the possible hindrance of interaction between the system and target cells (Suk et al., 2016). The clearance of these PEGylated liposomes is significantly increased by an emergent immune response to PEG, with approximately one quarter of blood donations containing antibodies against

PEG from previous exposure (Armstrong et al., 2007; Batrakova and Kim, 2015). In this respect, exosomes can be ideal nanocarriers for drugs due to their overall biocompatibility and reduced toxicity. The advantages of using them as vehicles include good tolerance due to their ubiquitous presence and similarity in structure and composition to cell membranes (Bang and Thum, 2012). Exosomes can penetrate deep in tissues and evade the immune system, good characteristics which allow them to deliver their therapeutic cargo directly to the cellular compartment. Exosomes from DCs were able to transfer peptide loaded MHC class I & II complexes to modulate cell response. Some exosomes are also capable of evading the immune system due to their size (Hood, 2016). Exosomes can seek out target tissues, making them ideal for targeting specific organs and tissues. Hood et. al. demonstrated this intrinsic ability to home in on targets by showing the way in which melanoma exosomes target sentinel lymph nodes (Hood et al., 2011).

Amenability to membrane modification can be a highly desirable attribute especially when targeting a specific cell type. The development strategy for targeted exosome-based delivery systems is based on the use of cell strains engineered with special plasmid vectors that encode fusion proteins. Fusion proteins basically consist of an extravesicle transmembrane domain and a targeting domain.

6.2. Delivery of drugs

Tian et al. (Tian et al., 2014) engineered mouse immature dendritic cells using protein (Lamp2b) fused to αv integrin-specific iRGD peptide. Immature DCs (imDCs) were chosen to produce the necessary exosomes to reduce immunogenicity and toxicity, as these cells are devoid of T-cell activators like MHC I, II and CD 86. The resulting exosomes were loaded with doxorubicin and were capable of targeting αv integrin expressing cancer cells. The imDCs were engineered to express exosomal membrane protein (Lamp2b) fused to αv integrin-specific iRGD peptide, thus targeting tumors. The study reported significantly lower cardiotoxicity and higher antitumor activity

when compared to the free drug (Tian et al., 2014).

The blood brain barrier (BBB) restricts delivery of therapeutic agents to the brain. It is composed of densely packed endothelial cells that provide a physical and biochemical barrier that prevents the entry of toxic compounds into the brain, preserving homeostasis. It also restricts the delivery of most drugs. In a recent study, exosomes derived from brain cells that expressed brain specific surface proteins, which allowed them to pass through the BBB and deliver drugs on the other side, were investigated. Exosomes isolated from brain endothelial cell line bEND.3 were loaded with rhodamine, doxorubicin or paclitaxel through mixing and incubation. When these exosomes were administered to glioblastoma cell, all the three agents were delivered to the cells. However, the exosomes loaded with doxorubicin showed higher activity than paclitaxel. The study further reports that a significant reduction in tumor size in the brain of zebrafish was observed, along with the inhibition of VEGF for the group that received exosome formulation, compared to the free drug group (Yang et al., 2015).

Exosomes separated from bovine milk were isolated using differential centrifugation. Then they were loaded with chemopreventive agent withaferin A and used against breast and lung cancer models. Two formulations were administered intraperitoneally to mice, the first one only containing withaferin and other with exosomal witheferin. A significant higher efficacy was observed with the drug loaded into exosomal formulation than with the free drug. The authors also reported good tolerance of the milk exosomes, with no adverse inflammatory or immune events observed (Munagala et al., 2016).

6.3. Delivery of protein and small RNAs

Parkinson's disease (PD) is a neurodegenerative condition, one of the quickest growing in developed countries, and often linked to microglia activation brain inflammation, and neurotoxic secretory activity, including increases in reactive oxygen species (ROS). Oxidative stress and

neudegeneration in PD may be attributed to decreasing catalase, redox enzymes and other antioxidants, and hence the delivery of potent antioxidant catalase can be instrumental in therapy. Haney et al. found that exosomes produced by monocytes and macrophages avoid entrapment in mononuclear phagocytes and further facilitate the delivery of incorporated drugs to the targeted cells of the neurovascular unit, ultimately increasing therapeutic efficacy of the drugs. The authors used several methods *ex vivo* to incorporate catalase (MW 240 K) into exosomes. Following intranasal administration, the study reported significant amount of catalase in the brain of PD mouse. The ExoCAT formulation interacted with target cells, delivering the payload to the neighboring neurons and providing significant levels of neuroprotection in the model used (Haney et al., 2015).

Another study in this direction was conducted by Alvarez-Erviti and coworkers, in which they genetically engineered DCs obtained from mice by fusing the well-characterized exosomal membrane protein; Lamp2b to neurospecific peptide RVG, as shown in Figure 2. Exosomes produced by these cells expressed RVG peptides on their surface which provided active targeting towards neuronal cells in the brain. When siRNA against BACE1, which is involved in the formation of plaque in Alzheimer's disease, was loaded in the exosomes, different parts of the brain showed specific gene silencing activity in the targeted neurons *in vivo* (Alvarez-Erviti et al., 2011).

Ohno et al. (Ohno et al., 2013) successfully targeted breast cancer cells that were overexpressing EGFR by using exosomes loaded with miRNA engineered using human embryonic kidney cells, expressing a transmembrane domain of platelet-derived growth factor receptors, fused either to epidermal growth factor receptor-binding peptides (GE11) or epidermal growth factors (EGF) (Ohno et al., 2013).

Glioblastoma multiforme (GBM) is a cancer of the central nervous system, and is the most widespread and lethal of this kind. It resists alkylating agents and antineoplastic treatments due to upregulated adenosine triphosphate–binding cassette drug efflux pumps. Chemoresistance and

functional drug efflux can be regulated by the use of microRNAs (miRs). Exosomes derived from mesenchymal stem cells (MSC) were investigated for providing GBM cells with functional anti-miR-9. The authors reported intracellular transfers between MSC and GBM cells. Delivering anti-miR-9 to resistant GBM cells was able to reverse the expression of the multidrug transporter, sensitizing these cells to temozolomide (TMZ), as demonstrated by increases in the activity of caspase and the death of the cells. The authors reported that inhibition of miR-9 by anti-miR decreases the expression of the drug transporter gene MDR1, leading to an increased sensitivity of the GBM cells towards TMZ (Munoz et al., 2013).

Exosomes can be used for anticancer vaccines to elicit immune responses via MHC class I and II molecules on CD8+ or CD4+ T cells, or through Mart1 pathway in patients with high-grade melanoma, or via the NK cell response (Inamdar et al., 2017). Several clinical trials around the world are in Phase I and II of testing on cancer patients, and the results look promising (Vlassov et al., 2012).

6.4. Delivery of natural compounds

Natural agents, such as dietary polyphenols, are able to modulate the content and therefore the function of exosomes. In 2007, Zhang et al. (Zhang et al., 2007) studied the effects of six different polyphenols (i.e. curcumin, genistein, quercetin, calycosin, biochanin and baicalein) on the restoration of the cytotoxicity of natural killer (NK) cells, inhibited by mouse mammary tumor exosomes. Turmeric exerts an wide range of bioactivities such as antioxidant, anti-protozoal, anti-venom activities, anti-microbial, anti-malarial, anti-inflammatory, anti-proliferative, anti-angiogenic, anti-tumor and anti-aging properties (Amalraj et al., 2017). TS/A tumor cells have been treated with genistein, curcumin, and baicalein reverting the suppression of the NK cells' cytotoxicity at a dose of 1 μM, and curcumin was found to provide the strongest restorative effect on NK cell function. Moreover, a wide range of curcumin concentrations yielded a concentration-

dependent increase in NK activity, down to concentrations as low as 200 nM. Furthermore, curcumin treatments were found to reduce inhibition of p-Stat5 and Jak3 in NK cells, as shown in Figure 3. This mechanism could be the basis of curcumin's capacity to promote NK cytotoxicity. In 2011, Zhuang et al. (Zhuang et al., 2011) delivered curcumin-loaded exosomes through a nasal route, and studied their effects on inflammatory diseases of the brain. They induced microglia inflammation, treating mice with lipopolysaccharide (LPS) and then immediately administering curcumin-loaded exosomes. The numbers of inflamed microglial cells were found to be reduced after 2 hours, along with an increase in apoptotic events compared to a control group. They also demonstrated that intranasal administration of exosomes containing curcumin reduced inflammation-induced experimental autoimmune encephalomyelitis in mice. Exosomes loaded with curcumin (CUR-EXO) were also active in mitigating brain endothelial cell dysfunction. Kalani et al. (Kalani et al., 2014) treated mouse brain endothelial cells with 7.5 micromol/L of curcumin, isolating exosomes after 72 h. Subsequently, endothelial cells were cultured in the presence of homocysteine, an agent capable of disrupting the blood brain barrier, or with both homocysteine and CUR-EXO. The results showed decreased oxidative stress for the samples co-treated with CUR-EXO. The coupled treatment was also found to increase claudin-5, occludin, ZO-1 and VE-chaderin levels, which have pivotal roles in the integrity of cerebral tight and adherent junctions. The activation of metalloproteinases (MMPs) is another sign of toxicity induced by homocysteine. It has been demonstrated that while homocysteine promotes the activity of MMP-9, curcumin primed exosomes normalize its activity. Osterman et al. (Osterman et al., 2015) found that curcumin loaded exosomes from pancreatic cancer cells induced apoptosis in recipient pancreatic adenocarcinoma cells, while curcumin negative exosomes increased the viability of these cancer cells.

6.5. Exosome cargo modified by natural compounds

Cafrán-Duque et al. (Canfrán-Duque et al., 2014) showed that HepG2 and THP-1 cells previously treated with U18666A increased their release of both exosomes and microvesicles when treated with curcumin at a concentration of 30 μM, ameliorating intracellular cholesterol traffic. They also studied the effects of curcumin on intracellular lipid accumulation caused by first and second generation antipsychotics. The results showed that curcumin decreased the content of lipids within the cell and that HepG2 cells, previously treated with antipsychotics, increased their secretion of cholesterol rich exosomes. Curcumin could thus be used as an adjuvant therapy to alleviate negative metabolic effects related to antipsychotic treatments (Canfrán-Duque et al., 2015). Taverna et al. (Taverna et al., 2016) suggested that curcumin inhibits the growth of chronic myelogenous leukemia (CML) cells through exosomal disposal of oncogenic miR-21. In fact, the intracellular levels of this miRNA decreased after 24 h treatment with 20 and 40 μmol/L of curcumin, while they increased in exosomes released to the culture medium. The intracellular reduction of miR-21 causes up-regulation of PTEN, a tumor suppressor protein, which is then able to antagonize the oncogenic pathway PI3K-AKT. In 2016, the same authors showed that the treatment of HUVEC cells with curcumin loaded exosomes derived from CML cells attenuated the motility and angiogenic properties of the endothelial cells. Furthermore, it has been demonstrated that the stabilization of cell junctions and vascular integrity is promoted by a decrease in expression levels of MARKS protein, one of the targets of miR-21 (Taverna et al., 2016). Treatment of breast cancer cells with docosahexaenoic acid (DHA) leads to increased small RNA exosomal content and the secretion of exosomal vesicles. Furthermore, the results showed that the DHA positive exosomes secreted were able to suppress tumor angiogenesis due to the content of microRNAs directed against pro-angiogenetic mRNAs, such as those coding for plasminogen activators, angiomotin like-1, neutropilin 1 and v-est avian erythroblastosis virus E26 oncogene homolog (Hannafon et al., 2015). Jang et al. (Jang et al., 2013) found that exosomes from murine breast

cancer cells treated with tea polyphenol epigallocatechin gallate (EGCG) induce a decrease in CSF-1 and CCL2, two growth factors for tumors promoting associated macrophages (M2). In addition, they promote the induction of the tumor inhibiting macrophages phenotype (M1). The molecular mechanism underlying this phenotype change is thought to be the up-regulation of miR-16 in exosomes after EGCG treatment, which in turn results in the negative regulation of nuclear factor-κB pathway frequently active in many inflammatory processes and cancers (Karin, 2009).

7. Conclusion

Recent breakthroughs made in the delivery of payloads by exosomes have attracted considerable attention of researchers worldwide. There is increasing evidence supporting the role of exosomes as a reliable delivery system. Several proof of concept studies have engineered exosomes to target specific cells and organs, delivering appropriate therapeutic agents. The great variability of the substances that can be loaded into exosomes and their unique properties, due to their biogenesis, makes exosomes powerful drug delivery vehicles. Nevertheless, to create a commercial exosomal drug delivery system that is superior to synthetic carriers, several complexities and obstacles have to be overcome. Some of the issues that needs to be considered for the therapeutic use of the exosomes include the procedure for their loading, selection of exosomes sources, improvement in purification, use of targeting peptides, stability of loaded agents and the formulation, pharmacokinetic properties and effects of modification of the exosome surface. In particular, the methods to load exosome with the desired cargo, constitutes a key point for the therapeutic success. There are different methods that can be applied and they could vary on the basis of the chemical/physical characteristic of the therapeutic cargo. Among different methods, there are electroporation, chemical-based transfection, transfection of the cell that will produce exosomes and the simple incubation of the exosomes with the molecule chose as cargo. As far as curcumin is concerned, given its lipophilic and low molecular weight, it is sufficient to co-incubate exosomes at

room temperature to promote the loading. Nevertheless, specific studies focused on the procedure for the loading into exosomes of other phytochemicals have to be performed.

In conclusion, considering that phytochemicals have emerged as effective anticancer agents, and many natural products are currently being evaluated for efficacy and are in various phases of clinical trials, exosomes could be considered as remarkable delivery vehicles for these phytochemicals.

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Legends to figures:

Figure 1. Structural composition of exosomes.

Figure 2. Design of targeted exosomes. Exosomal surface protein Lamp2b (blue) is fused with RVG (red).

Figure 3. Curcumin mediated increase in the phosphorylated STAT5.

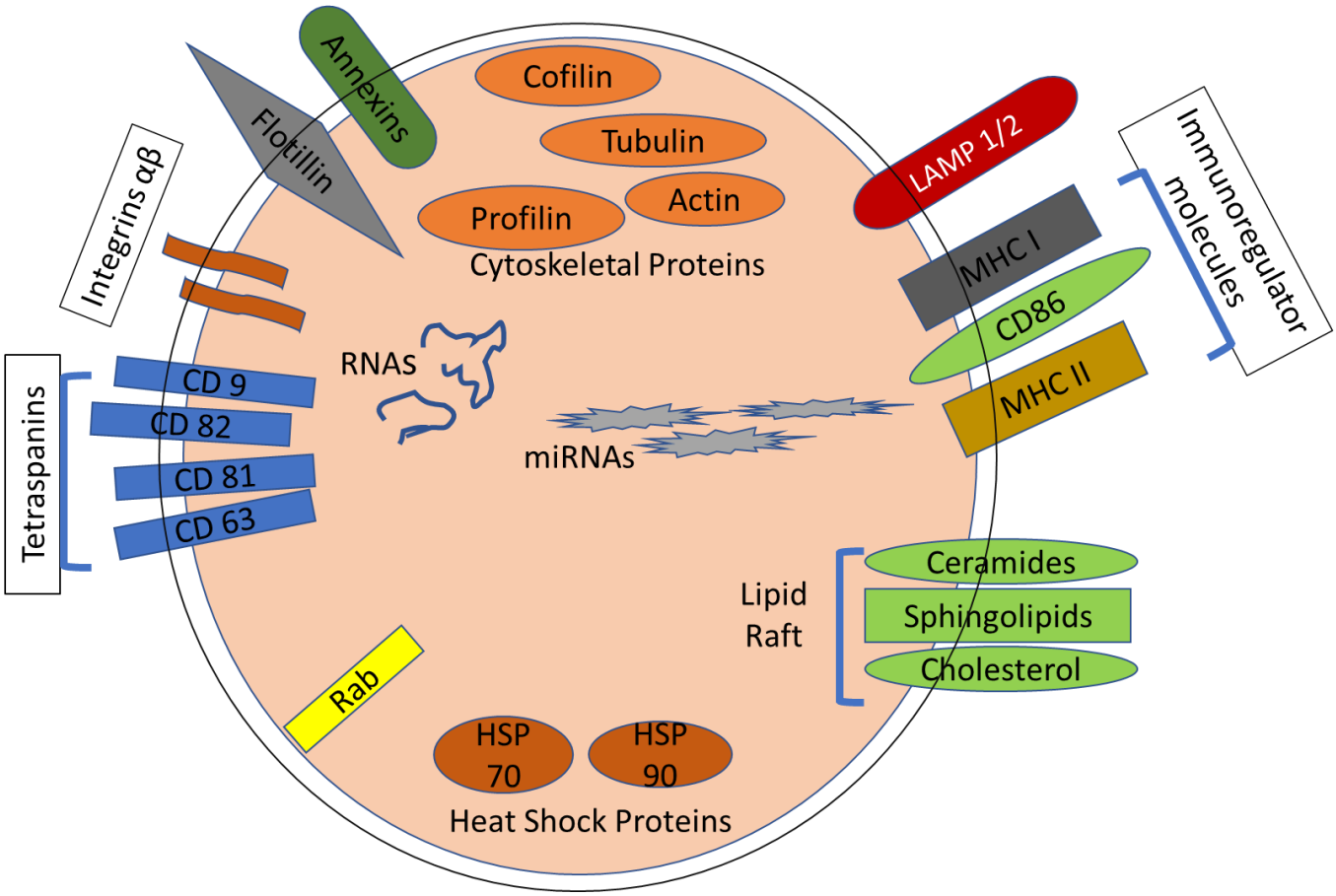

Figure 1.

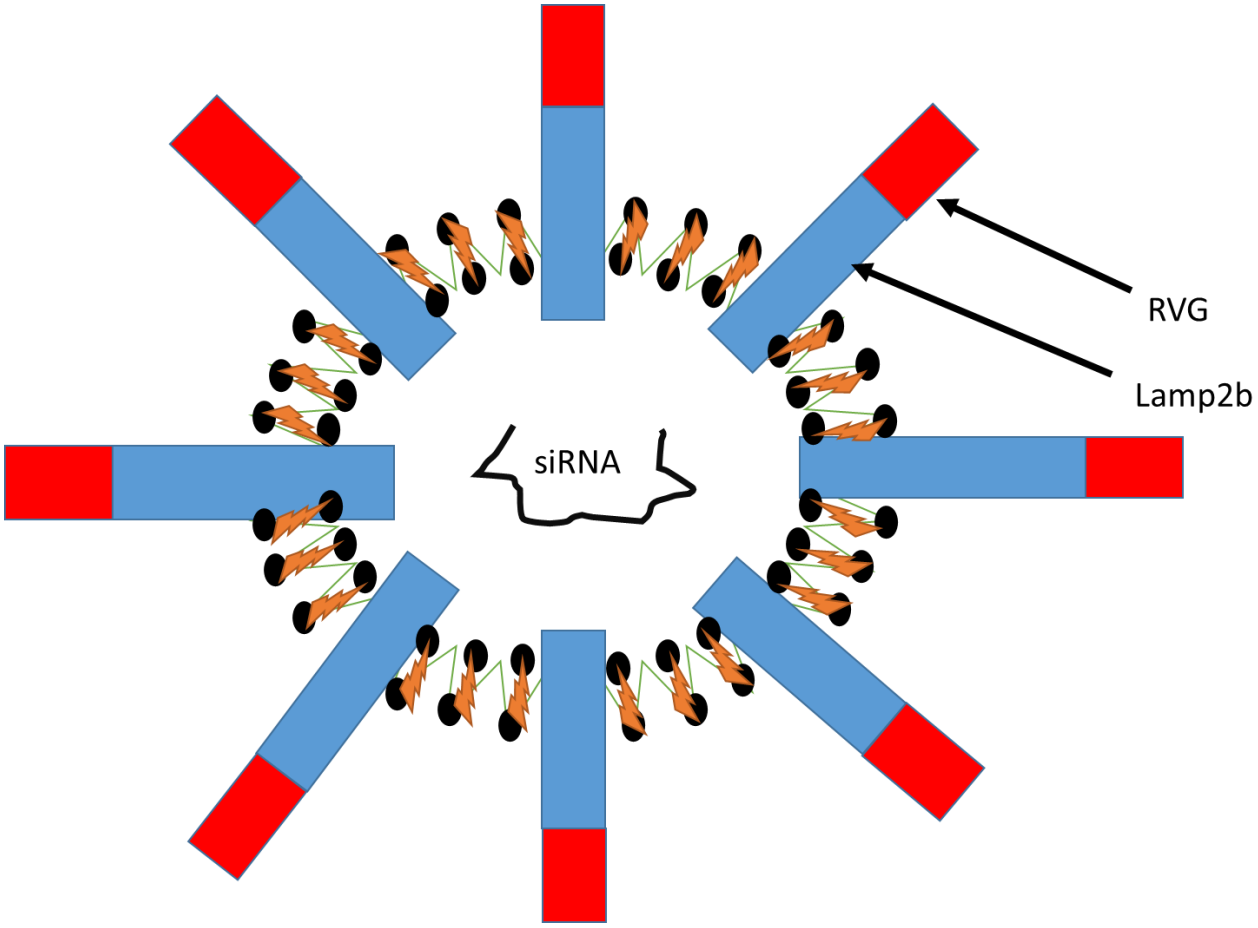

Figure 2.

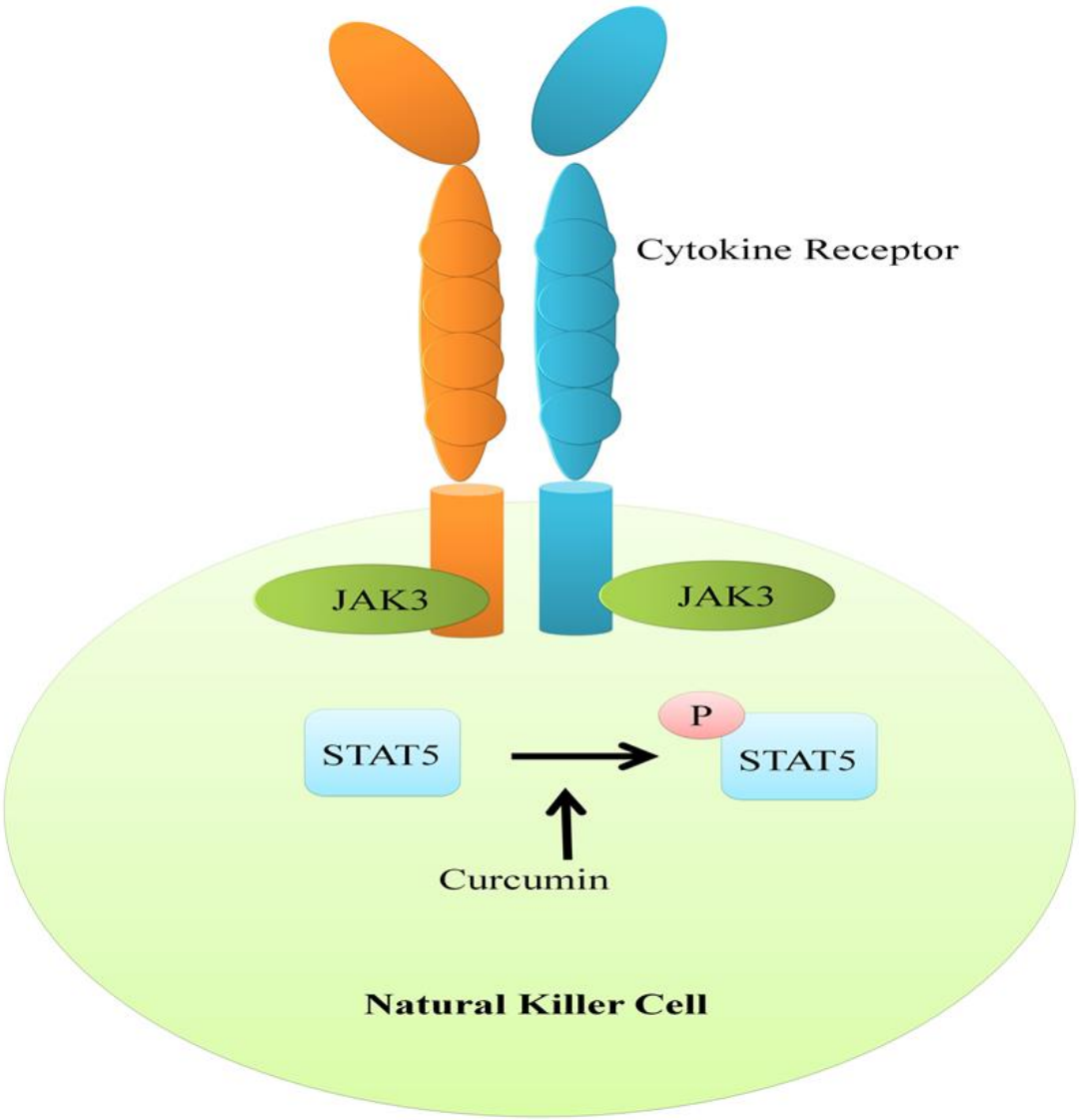

Figure 3.

Highlights

- Cells release extracellular vesicles into extracellular compartments.
- Natural compounds show health benefits impacting on a number of human diseases.
- Exosomes can be used as delivery systems for natural compounds.