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Review

A comprehensive overview of exosomes as drug delivery vehicles — Endogenous nanocarriers for targeted cancer therapy

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ABSTRACT

Exosomes denote a class of secreted nanoparticles defined by size, surface protein and lipid composition, and the ability to carry RNA and proteins. They are important mediators of intercellular communication and regulators of the cellular niche, and their altered characteristics in many diseases, such as cancer, suggest them to be important both for diagnostic and therapeutic purposes, prompting the idea of using exosomes as drug delivery vehicles, especially for gene therapy. This review covers the current status of evidence presented in the field of exosome-based drug delivery systems. Components for successful exosome-based drug delivery, such as choice of donor cell, therapeutic cargo, use of targeting peptide, loading method and administration route are highlighted and discussed with a general focus pertaining to the results obtained in models of different cancer types. In addition, completed and on-going clinical trials are described, evaluating exosome-based therapies for the treatment of different cancer types. Due to their endogenous origin, exosome-based drug delivery systems may have advantages in the treatment of cancer, but their design needs further refinement to justify their usage on the clinical scale.

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Abbreviations: AAV, adeno-associated virus; Aex, ascites-derived exosomes; BACE1, beta secretase 1; BAP-TM, biotin acceptor peptide-transmembrane domain; CAM, cell adhesion molecule; CD, cluster of differentiation; CD, cytosine deaminase; Dex, dendritic cell-derived exosomes; EGFP, enhanced green fluorescent protein; EGFR, epidermal growth factor receptor; ESCRT, endosomal sorting complex required for transport; GAPDH, glyceraldehyde triphosphate dehydrogenase; GBM, glioblastoma multiforme; GTP, guanosine triphosphatase; HCV, hepatitis C virus; HEK-293, human embryonic kidney cell line 293; HeLa, Henrietta Lax; IDH1, isocitrate dehydrogenase 1; LAMP, lysosomal-associated membrane protein; LFA-1, lymphocyte function-associated antigen 1; LPS, lipopolysaccharide; MHC, major histocompatibility complex; miRNA, microRNA; MRI, magnetic resonance imaging; mRNA, messenger RNA; MSC, mesenchymal stem cell; MVB, multivesicular body; PDGFR, platelet-derived growth factor receptor; RAB, Ras-related protein; RNA, ribonucleic acid; RVG, rabies viral glycoprotein; shRNA, short hairpin RNA; SPION, superparamagnetic iron oxide nanoparticle; STAT, signal transducer and activator of transcription; UPRT, uracil phosphoribosyltransferase

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1. Introduction

Exosomes denote a family of nanoparticles with a diameter in the range of 30–120 nm that are secreted by most cell types of the body [1,2]. They can be isolated from several types of extracellular fluids including blood, urine, amniotic fluid, saliva, and cerebrospinal fluid [2–4]. Exosomes were first described by Trams et al. and later substantiated by Johnstone et al., who observed high levels of transferrin binding onto small particles (exosomes), while the same binding activity was lost on parent cells [5–8]. These data suggested that exosomes were used by the cells as a major route of excretion, which would allow them to dispose of any unused or harmful RNA and proteins, in case no appropriate lysosomal degradation systems were available [6–8]. Exosomes are now regarded as a distinct cellular entity specifically capable of carrying cargos like RNA, proteins, lipids etc. to be shared between cells [2,9].

1.1. Biogenesis of the exosome

Inside the cell, exosomes are initially produced by a process of invagination into endosomal membranes to create multivesicular bodies (MVBs) (Fig. 1) [10]. This distinguishes the exosomes from the shedding microvesicle that forms via direct budding of the cell membrane [2,7]. The formation of MVBs in exosome genesis suggests some similarities with the MVBs formed during lysosome formation, since lysosomal surface proteins, such as LAMP and CD63, are present in the exosomal membrane [10–12]. The formation of exosomes and sorting of cargo into them entails a collection of proteins, the so-called endosomal sorting complex required for transport (ESCRT), which is also crucial for lysosome formation [13,14]. The ESCRT machinery encompasses four major protein complexes, ESCRT-0–ESCRT-III. Together with a number of accessory proteins, the ESCRT machinery is known to favor endosomal sorting of ubiquitinylated proteins for secretion in nanoparticles such as exosomes [13]. Distinct from that of lysosome formation, exosome formation in some cell types can also be dependent on lipid raft-like domains on the endosome membrane rich in the sphingolipid, ceramide [15].

Secretion of exosomes is achieved by fusion of the MVB and the cell membrane (Fig. 1), which is thought to be dependent on several Rab GTPase proteins including RAB27A, RAB27B, RAB11 and RAB35 [16,17]. This secretion can be inhibited experimentally by treatment with the ceramide biosynthesis inhibitor, GW4869 [15,18]. Exosome release is increased in highly proliferative cells (such as mesenchymal stem cells (MSCs)), and the large exosome formation capability of these cells can be experimentally induced without mediating any physiological changes to the resulting exosomes by transfection with the MYC gene [19,20]. Interestingly, detachment of cultured breast cancer cells from various substrates rapidly increases the release of exosomes, with significant effects on attachment and spreading; cellular processes, which could clinically favor metastasizing cancer [21].

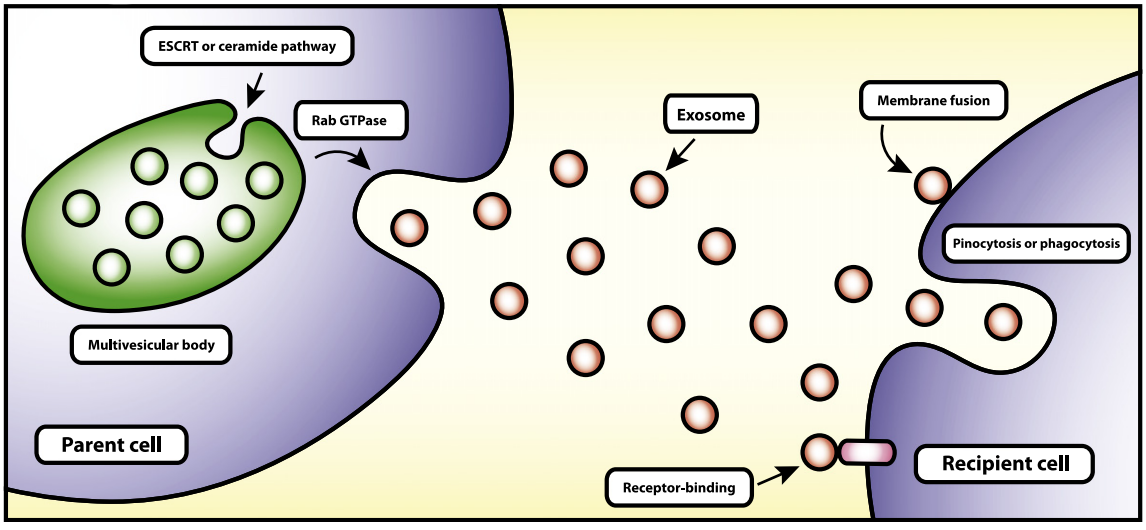


Fig. 1. Biogenesis of exosomes. The formation of exosomes starts by inward budding of the endosomal membrane to create multivesicular bodies (MVBs) in the cell cytoplasm. This process is dependent on either the endosomal sorting complex required for transport (ESCRT) machinery or the sphingolipid ceramide. Rab GTPase-dependent fusion of the MVBs with the parent cell membrane releases the produced exosomes to the extracellular space, where they can interact with recipient cells. The delivery of the exosomal cargo to the recipient cell can occur by ligand–receptor interaction, pinocytosis/phagocytosis or fusion with the cell membrane.

1.2. Structure and contents of exosomes

The surface of exosomes is characterized by the presence of multiple families of proteins, such as tetraspanins (CD63, CD81, CD9), heat shock proteins (Hsc70), lysosomal proteins (Lamp2b) and fusion proteins (CD9, flotillin, Annexin) [22]. The tetraspanins have received a lot of attention, because the trinity of CD63, CD81 and CD9 has been widely used as exosome markers. However, the possible existence of single exosome-specific proteins has yet to be disclosed [23]. Most of the proteins described above are exclusively expressed on the exosomal surface, which distinguish them from other endogenously derived nanoparticle-like structures, such as shedding microvesicles and apoptotic bodies [24]. This enables their utilization as exosome markers. Exosomes also contain large amounts of intraluminal proteins to be shared between cells as a way of paracrine signaling. Currently, more than 11,000 proteins have been identified in association with exosomes [25].

Valadi et al. were the first to report on the presence of RNA inside exosomes derived from mouse and human mast cells. Several types of RNA (especially mRNA and miRNA) could be isolated from the exosomal compartments, and the mRNA was functional in generating proteins in an in vitro translation assay [26]. Transfer of exosomal mRNA, miRNA and proteins between different glioblastoma multiforme (GBM) cell populations resulted in increased proliferation, since the components or cargo of the exosomes reflected the malignant potential of the parent cells [27]. In a complementary study, rat 9L gliosarcoma cells and human U87MG cells were engineered to express a *Caenorhabditis elegans*-specific miRNA (cel-miR-67). Co-culturing these cells with 9L and U87MG cells engineered to express a luciferase-reporter with a complementary sequence to cel-miR-67 gave rise to functional transfer of this specific miRNA between different cell populations, hence signifying a role for exosomes in paracrine signaling between cancer cells [28].

1.3. Physiology of the exosome

Exosomes have been endorsed with a vast array of functions; especially their role in intercellular communication, which has received much attention as a basic characteristic, transporting RNA and proteins between cells [24,26,29–31]. Transport of exosomes increased the expansion of the hematopoietic stem cell pool via uptake of embryonic stem cell-derived microvesicles, regenerated kidney epithelium and heart muscle tissue after ischemic injuries, and initiated coagulation due to their content of tissue factor [29,32–34]. Neurons release exosomes from the soma and dendrites, proposed as a possible way for pathophysiologically important components to be shared, leading to neurodegeneration [35]. The neuronal release of exosomes was modulated by synaptic activity, adding great complexity to the role of neuronal derived exosomes [35,36].

The release of exosomes by immune and cancer cells emphasizes important aspects of these small extracellular vesicles in the human physiology [37,38]. Exosomes are significant for the normal antigen-presentation by dendritic cells, B- and T-cell activation and immune cell effector functions (reviewed in Théry et al.), and they may play a significant role in the normal immune response against malignantly transformed cells [37]. Conversely, cancer cells were shown to escape the immune system, thus retaining their uncontrolled growth potential due to mechanisms mediated by exosomes [39–41]. Exosomes isolated from the serum of patients suffering from oral squamous cell carcinoma possessed similar surface protein markers as the parent cancer cell, e.g. they were highly enriched in Fas ligand. Incubation of these cancer cell-derived exosomes and T-lymphoblasts from these patients induced apoptosis of the T-cells due to interaction with the Fas receptor [40]. Cancer cell-derived exosomes were also shown to induce expansion of regulatory T-cells in vitro, hence pointing towards another important factor in the immune escape of cancer cells [41].

Cancer cells share genetic components with each other via exosomes to increase their malignant potential, an exchange shown to be dependent on so-called invadopodia and heparan-sulfate proteoglycans [42–44]. These tumor cell-derived exosomes are able to improve the tumor niche, facilitating both tumor expansion and cancer cell metastasis [45–47]. For example, exosomes derived from hypoxic regions of a tumor or from cancer cells grown under hypoxic culture conditions induced proliferation, invasion and especially angiogenesis in cancer cells or tumor-associated endothelial cells [48–50]. Some cancer cell-derived exosomes possess a nanofilamentous network, which facilitates interaction with the cell membrane and increases the cellular exosome uptake [51]. Such special characteristics of cancer cell-derived exosomes could possibly be a source of inspiration in the design of efficient exosome-based drug carriers for use in cancer treatment.

Cancer cell-derived exosomes might also be beneficial as biomarkers for detecting cancer growth at early stages due to their change in characteristics compared to those secreted by non-malignant cells [47,52]. Serum from nine GBM patients was significantly different from normal controls with respect to the content of exosomal RNA [53]. This presence of altered exosomes being present in the circulation during malignant disease has led to the discovery of several potential biomarker candidates for cancer diagnostics, such as exosome-borne EGFR, IDH1 and c-myc mRNA [54–57]. For example, an on-going phase I clinical trial is now investigating the potential of using exosomes in the diagnosis and prognosis of patients with gastric cancer (The EXO-PPP study, NCT01779583).

2. Aim

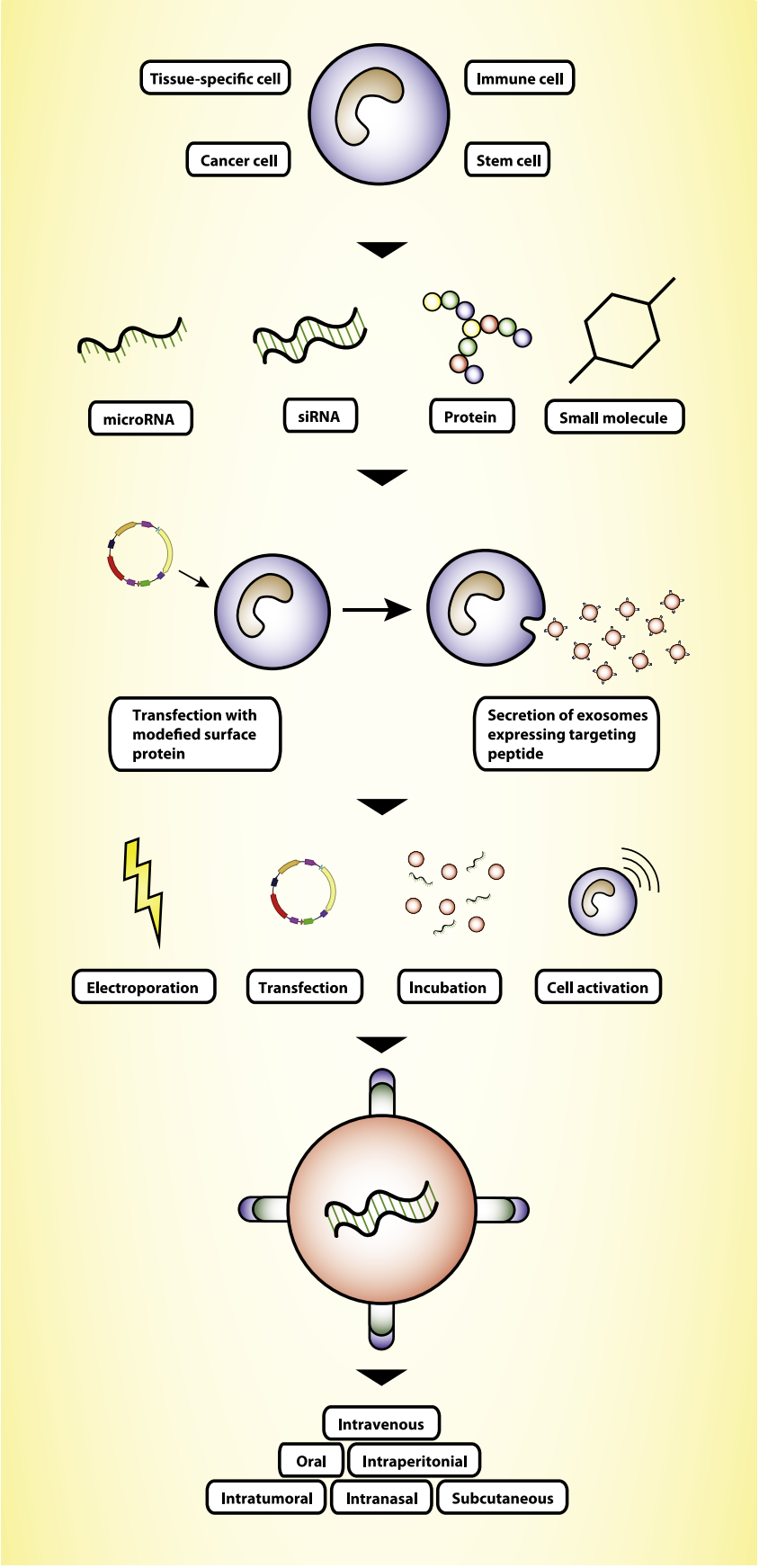
The aim of this review was to present a comprehensive overview of all studies pertaining to exosomes in drug delivery and to evaluate the future relevance of exosomes in cancer treatment. Special focus has been placed on identification of components enabling successful exosome-based drug delivery, including choice of donor cell, therapeutic cargo, use of targeting peptides, loading method, and routes of administration to fully unravel the potential of exosomes as drug delivery vehicles for future clinical use.

3. Methodology and delimitations

A PubMed database search on exosomal drug delivery (typed: “exosome AND drug delivery”) was performed (date of last search entry: January 9, 2014) revealing a total of 64 papers, which were analyzed for reports on exosome delivery of specific therapeutic cargos. Studies investigating the potential of unmodified exosomes for the treatment of specific diseases were not included in this review. Furthermore, the reference lists of all available review papers commenting on exosome drug delivery were assessed for any studies that were not revealed in the PubMed search. A total of 24 studies were found to meet the inclusion criteria by investigating the potential of modifying exosomes to deliver therapeutic cargo, and thus included in this review. These studies were sorted and summarized with respect to relevant information on choice of donor cell, therapeutic cargo, use of targeting peptides, loading method, administration routes etc. (presented in Supplementary Table 1).

4. Components of successful exosome-based drug delivery

In recent years, several studies have highlighted situations, in which an exosome-based drug delivery system has improved disease conditions, including studies performed on different cancer models. Development of an exosome-based drug delivery system requires that different components must be appropriately chosen and controlled in order to achieve the correct functionality and efficacy. These components are discussed in the following sections and summarized in individual tables and in Fig. 2.


Tissue-specific cell
Immune cell
Cancer cell
Stem cell
microRNA
siRNA
Protein
Small molecule
Transfection with modefied surface protein
Secretion of exosomes expressing targeting peptide
Electroporation
Transfection
Incubation
Cell activation
Intravenous
Oral
Intraperitonial
Intratumoral
Intranasal
Subcutaneous

4.1. The choice of donor cell type depicts specific characteristics of the drug-carrying exosome

An initial requirement before reaching an efficient exosome-based drug delivery system is the optimal choice of donor cell type. The cell should produce exosomes devoid of any immunologically stimulating activity to prevent inflammatory responses after administration, while also being stable in the circulation to allow for sufficient time to deliver their cargo. Until now, numerous cell types have been used as exosome factories, with some cell types used more frequently than others. These include model cell lines such as HeLa and HEK-293, which were used in seven out of the 24 studies included in this review (Supplementary Table 1) [58–64]. In addition, different types of murine melanoma cell lines (B16-F10, B16-BL6 and B16-F1) were used as donor cells in three studies [65–67].

Immature dendritic cells have favorable properties with respect to immunogenicity due to their special surface protein composition, which make them relevant exosome donor cells [68]. In fact, exosomes from these cells have now been used in two studies, in which they were modified to express a targeting peptide on their surface, and shown to carry different types of therapeutic cargo [69,70]. However, the workload required to upscale the production of these cells and the resulting yield of exosomes are currently not suitable for clinical use [70].

MSCs were used as exosome donor cells in three studies [71–73]. The approach of using MSC exosomes for drug delivery is interesting because MSCs are known to produce large amounts of exosomes, suggesting that these cells may be efficient for exosome production in a clinically applicable scale [19,20]. However, as the cancer-stimulating properties of MSC exosomes and microvesicles are disputed, care should be taken when using these cells as exosome donors in cancer treatment [74,75]. In this regard, it should be stated that MSC exosomes used for drug delivery to GBM xenograft tumors revealed a reduction in tumor size [73]. Thus, a combination of the tissue-specific targeting achieved in some studies (see Section 4.4) and the exosome production efficiency from MSCs could be an interesting approach to pursue in obtaining the most appropriate exosome factory.

4.2. Exosomes deliver a variety of therapeutic cargos

Exosomes should be able to carry a substantial amount of therapeutic cargo to qualify as drug delivery vehicles. A variety of cargos have now been shown to exhibit therapeutic effect after exosome-based delivery to particular tissues. The majority of studies exploit an important physiological characteristic of the exosomes, i.e. the transfer of interfering RNAs, while fewer studies investigate the potential of loading other types of therapeutic cargo into the exosome particles (Table 1).

4.2.1. siRNA

Three studies examined small interfering RNAs (siRNAs) as therapeutic cargo, because these RNA molecules have a substantial potential in gene-based therapy [62,64,69,76,77]. Exosome-based delivery of interfering RNAs is of major interest, because the stability of these RNA molecules is very low due to their rapid degradation in the systemic circulation [78,79]. Exosomes derived from both cells and plasma could effectively deliver MAPK1-siRNA to recipient peripheral blood mononuclear cells resulting in a specific gene knockdown in vitro [62]. A similar methodology was used to deliver RAD51- and RAD52-siRNA to induce both gene knockdown and decrease fibrosarcoma cell viability and proliferation [64]. Downregulation of the mRNA expression of the housekeeping gene, GAPDH, and the Alzheimer's disease-associated gene, BACE1, was observed specifically in neurons after targeted delivery of siRNA-enriched exosomes [69]. Other studies have provided insight to the use of short hairpin RNAs (shRNAs) and the so-called self-delivering RNAs as therapeutic cargo in exosomes [59,64]. For example, shRNAs against a viral entry receptor and hepatitis C virus (HCV) replication machinery were stably transfected into several cell types, and the resulting shRNA-loaded exosomes mediated a decrease in HCV infection of liver cells [59].

4.2.2. MicroRNA

Since exosomes naturally carry miRNAs, a therapeutic application of this characteristic seems logical, as illustrated in the number of studies applying the approach for different disease models [26]. Exosome-encapsulated miR-150 was shown to decrease endothelial cell migration and to mediate suppression of effector T-cells, while 293T and T-cell expression of miR-122 increased several-fold after being cultured in the presence of exosomes in conditioned medium from miR-122-transduced 293T cells [59,80,81]. MSC exosomes enriched in miR-133b increased the level of neurite outgrowth in vitro, suggesting these MSC exosomes to become a potential treatment in brain ischemia [71]. In addition, miR-214 could be shuttled via exosomes to hepatic stellate cells resulting in a decreased expression of CCN2, a gene known to be important in regulating liver fibrosis [82,83].

Most cancer types are characterized by a dysregulated expression profile of numerous miRNAs, as recently reviewed for GBM [84,85]. In this regard, MSC-derived exosomes with a high expression of tumor suppressor miRNA, miR-146b, inhibited tumor growth in a xenograft model of GBM, thereby underscoring the relevance of both exosome-based drug delivery and miRNA-based therapy [73,86]. MSC exosomes delivered anti-miRs for the knockdown of the oncogenic miRNA, miR-9, in GBM cells in vitro, increasing the GBM cell sensitivity to chemotherapeutic treatment with temozolomide [72]. This study also provided evidence that exosomes were responsible for most of the communication between MSCs and GBM cells, as the transfer of fluorescently labeled anti-miRs could still be achieved after blocking the formation of gap junctions [72]. Exosome-delivered tumor suppressor miRNAs, miR-143 and let-7a, inhibited growth of prostate and breast cancer in vivo, respectively [60,61]. No adverse effects were observed in normal prostatic epithelial cells after treatment with exosome-encapsulated miR-143 [60].

The above-mentioned studies provide an illustration as to how the combined effects of miRNA- and exosome-based therapies may be valuable, especially for the future treatment of cancer. However, in order to secure reproducibility of miRNA-bearing exosome preparations, elucidation of the mechanisms by which miRNAs are normally loaded into exosomes is required. This could include further investigation of the so-called EXO-motifs, which have been proposed to direct specific miRNAs towards secretion from the cell via exosomes [87]. The concept should also be tested in other cancer models to validate its general applicability in a cancer treatment regimen.

4.2.3. Encapsulation of other therapeutic compounds

Even though the vast majority of exosome-based drug delivery studies investigate the therapeutic transfer of interfering RNAs, other types of therapeutic cargo can be loaded into these particles. Of particular interest to the field of exosome-based cancer therapies are two studies that recently provided evidence to the possibility of loading chemotherapeutics into exosomes, namely doxorubicin. Doxorubicin loaded into exosomes or exosome-mimetic nanovesicles inhibited the growth of breast and colon adenocarcinoma xenograft tumors in vivo [70,88]. In addition, by targeting immature dendritic cell exosomes directly to

Fig. 2. Components of successful exosome-based drug delivery. Exosome-based drug delivery requires the correct choice of donor cell type for the specific application. The exosomes from the chosen cell type should ideally possess specific characteristics, such as stability in the circulation, and be devoid of any immunogenicity. The therapeutic cargo can include different types of interfering RNAs, proteins or even chemotherapeutics, when used to treat cancer. To achieve effective treatment without major side effects, the exosomes should be modified to express a targeting peptide on their surfaces. The method used to load the exosomes should be chosen carefully, to avoid changes to the therapeutic cargo that might decrease the efficiency of the treatment. Finally, the choice of administration route depends on the specific application of the exosome drug delivery system.

the tumor tissue, the efficacy of doxorubicin was greatly enhanced. The enhanced efficacy was combined with significantly less adverse effects on major organ systems, especially the heart, which are normally induced by doxorubicin treatment, implying that delivery via exosomes might decrease the major downside of this chemotherapeutic drug [70,89].

Based on prior evaluations of the stability and anti-inflammatory effects of exosome-encapsulated drugs, the STAT3 inhibitor JSI-124 (cucurbitacin I) was loaded into exosomes and shown to decrease the tumor volume in a murine model of GBM [90–92]. HEK-293T cells were transfected with a vector construct consisting of cytosine deaminase (CD) fused to uracil phosphoribosyltransferase (UPRT) to produce exosomes enriched in this protein construct [63]. These exosomes were used as a co-treatment with the chemotherapeutic prodrug, 5-fluorocytosine, in an orthotopic model of schwannoma, to facilitate conversion to the active 5-fluorouracil and 5-fluoro-deoxyuridine monophosphate [63,93]. This co-treatment led to significant tumor cell apoptosis and tumor regression, further underscoring the potential of (protein)-loaded exosomes in the treatment of malignancy [63]. Furthermore, exosomes loaded with superparamagnetic iron oxide nanoparticles (SPIONs) may have potential in cancer diagnostics using MRI, and for therapeutic purposes using localized magnetic hyperthermia [65,94].

The pallet of different therapeutic cargos that until now have been loaded into exosomes demonstrates how these particles have potential as drug delivery vehicles. Most studies investigate this potential with regard to the normal physiology and function of an exosome (i.e. the delivery of miRNA, mRNA and protein), but interestingly, exosomes can also be exploited to deliver other substances such as chemotherapeutics. Therefore, it must be expected that future research will uncover new types of cargo to be delivered via exosomes, and possibly map the conditions suitable for a specific type of exosome-encapsulated cargo. Revealing the sorting and packaging mechanisms of exosomes may lead the way for purer exosome cargo, hereby avoiding any physiological side effects mediated by the endogenous cargo of a particular type of exosomes.

4.3. Methods for loading exosomes with therapeutic cargo

Successful delivery of substantial amounts of therapeutic cargo from exosomes highly depends on an efficient method of their loading [65,95]. The approaches utilized in the studies published to date include classical cell transfection methods like electroporation, simple incubation and the use of chemical transfection reagents, but more sophisticated methods like transfection or activation of the exosome donor cell have also been utilized (Table 2).

Table 1
Types of therapeutic cargo loaded into exosomes.

Study	Cargo
Interfering RNAs	
Shtam et al. [64]	siRNA against RAD51 and RAD52
Wahlgren et al. [62]	MAPK1 siRNA
Alvarez-Erviti et al. [69]	GAPDH siRNA & BACE1 siRNA
Pan et al. [59]	shNS5b, shCD81
Chen et al. [82]	miR-214
Bryniarski et al. [81]	miR-150
Zhang et al. [80]	miR-150
Katakowski et al. [73]	miR-146b
Kosaka et al. [60]	miR-143
Pan et al. [59]	miR-122
Xin et al. [71]	miR-133b
Ohno et al. [61]	Let-7a
Munoz et al. [72]	Cy5-anti-miR-9
Other types of therapeutic cargo	
Tian et al. [70]	Doxorubicin
Jang et al. [88]	Doxorubicin
Hood et al. [65]	Superparamagnetic iron oxide nanoparticles (SPION5)
Mizrak et al. [63]	Cytosine deaminase (CD) fused with uracil phosphoribosyltransferase (UPRT) and EGFP
Maguire et al. [58]	Adeno-associated viral vector
Zhuang et al. [90]	Curcumin & JSI-124
Sun et al. [92]	Curcumin

4.3.1. Electroporation

By applying an electrical field to a suspension of exosomes (or cells) and the therapeutic cargo of choice, pores are created into the lipid bilayer membrane, hereby facilitating the movement of cargo into the lumen of the exosomes [96]. In the key paper by Alvarez-Erviti et al. loading of siRNA into exosomes was achieved via this method of electroporation [69]. Several other studies also showed efficient uptake of the therapeutic cargo by use of electroporation with varying voltage settings in the range of 150–700 V, suggesting that the optimal parameters for exosome electroporation may vary between different donor cell types, such as monocytes, HeLa cells and immature dendritic cells [62,64,70]. In addition, the concentration of exosomes per electroporation ranges from 0.07 to 0.5 μg/μL [65,69,70,97].

Interfering RNAs are the compound most frequently loaded into exosomes, which corresponds well with the original applications of this method, and is a logical choice, since exosomes naturally carry interfering RNAs [26,96]. As previously described, delivery of interfering RNAs is of great interest for the treatment of different cancer types due to their known dysregulations in the expression of numerous miRNAs and mRNAs [77]. The quantitative efficiency of siRNA was evaluated in two studies using fluorescently labeled siRNAs for quantification, revealing that the uptake was around 25% of the total siRNA in the solution, and also that electroporation was superior to chemical transfection obtained using a commercial transfection reagent [62,69]. Uptake was also evaluated by investigating the functional effects on HeLa cells after addition of RAD51 siRNA-loaded exosomes, showing that these exosomes effectively decreased the cell viability [64].

Electroporation may be a favorable choice of loading method in a clinical setting since the parameters of the method can be easily controlled, but it could also be suspected to induce adverse effects on the integrity of the exosome particles or the therapeutic cargo. The level of efficiency in siRNA loading by electroporation was recently questioned by Kooijmanns et al., who presented evidence that the process of electroporation induces the formation of siRNA aggregates, which cannot be determined only by measuring the amount of fluorescence of the labeled siRNAs. In fact, the real efficiency of siRNA retention in exosomes after electroporation was below 0.05% [95]. Moreover, electroporation could induce aggregation of the exosome particles themselves, but with optimized electroporation parameters and utilization of so-called trehalose pulse media, the exosome aggregation was diminished, while still allowing for iron particle loading [65]. Finally, electroporation was used to load doxorubicin into targeted exosomes, showing that chemotherapeutics could be a relevant type of drug for loading into exosomes by this method, since the function of the drug was maintained [70].

4.3.2. Chemical-based transfection

Chemical-based transfection using commercial transfection reagents has been used only in two studies to load exosomes with siRNA [62,64]. The efficiency of siRNA loading into exosomes was lower for the *HiPerFect transfection reagent* when compared to electroporation, and even though *Lipofectamine 2000* could be used to load siRNA and decrease gene expression after delivery to recipient cells, the isolated effects of only the loaded exosomes could not be determined, since leftover micelles (with captured siRNA) may have been present in the resulting exosome preparation [62,64]. Therefore, chemical-based transfection seems inadequate as a method of efficient loading of siRNAs into exosomes for therapeutic purposes.

4.3.3. Transfection of exosome-producing cells

The most widely used approach for loading therapeutic cargo into exosomes is by transfecting the exosome donor cell to overexpress a certain gene product that the cell will package into the exosome lumen or membrane for secretion (Table 2) [61,73]. miRNAs were introduced into exosomes in several studies, using miRNA expression vectors or pre-miRNAs [59–61,73,82]. MSCs were transfected with a miR-146b expression vector, and the resulting miR-146b-expressing MSC exosomes mediated inhibition of cancer growth [73]. This study provided interesting evidence to the use of exosomes in cancer treatment, and substantiated the results from another study, where the exosomes, in addition to containing large amounts of let-7a, also were endowed with a targeting peptide to facilitate efficient delivery after intravenous injection [61,73]. High miR-214 expression in exosomes could be achieved by transfecting the donor cells with pre-miR-214, while anti-miRs could also be transfected to the donor cells in a similar way, and packaged into exosomes [72,82].

Two studies have shown that transfection can be used to overexpress a specific protein on the surface membrane of the exosome, while a third study showed that proteins could be loaded into the exosomes by transfection of the donor cells [63,67,98].

The process of donor cell transfection for loading of therapeutic cargo into exosomes is generally very effective, which is reflected in the large number of studies using this approach, together with the fact that the miRNAs loaded into exosomes by this method all contain the recently described EXO-motifs in their mature sequences [87]. Evidently, this method proved its relevance in in vivo experiments, and therefore may be one of the most appropriate methods for exosome loading at the moment. However, one must bear in mind that the cell engineering required to achieve both the presence of targeting peptide on the exosome surface, and loading of large amounts of cargo to the exosome lumen may be a laborious and time-consuming procedure. This is a problem for clinical application if the patient's own cells are to be used as exosome donors. Therefore, the development of non-autologous exosome factories generating non-immunogenic, targeted and loaded exosomes could be of great interest.

Table 2
Methods for loading therapeutic cargo into exosomes.

Study	Setup
Electroporation	
Tian et al. [70]	350 V, 150 μF
Hood et al. [65]	750 V/cm
Shtam et al. [64]	700 V, 350 ms, 20 pulses
Wahlgren et al. [62]	150 V, 100 μF
Alvarez-Erviti et al. [69]	400 V, 125 μF
Chemical-based transfection	
Shtam et al. [64]	Lipofectamine 2000
Wahlgren et al. [62]	HiPerFect Transfection Reagent
Incubation of exosomes with cargo	
Jang et al. [88]	37 °C
Bryniarski et al. [81]	37 °C
Zhuang et al. [90]	22 °C
Sun et al. [92]	22 °C
Transfection of exosome-producing cells	
Chen et al. [82]	LX-2 hepatic stellate cells
Katakowski et al. [73]	Mesenchymal stem cells
Mizrak et al. [63]	HEK-293T cells
Munoz et al. [72]	Mesenchymal stem cells
Pan et al. [59]	HEK-293T cells and Huh7 cells
Rana et al. [98]	BSp73AS adenocarcinoma cells
Ohno et al. [61]	HEK-293 cells
Maguire et al. [58]	HEK-293 cells
Kosaka et al. [60]	HEK-293 cells
Zhang et al. [80]	THP-1 monocytes
Cell activation	
Xin et al. [71]	Activation with brain extracts from MCAo rats
Zhang et al. [80]	Activation with LPS, OA/PA or AGE

4.3.4. Cell activation

While cell activation may not be the most appropriate choice of methodology for properly controlled exosome loading in clinical application, it does indeed shed light on an interesting perspective of exosome physiology. The process of cell activation as a means of achieving exosome loading was, however, only studied twice. THP-1 monocytes stimulated with three inflammatory stimulants revealed increased levels of miR-150 in the resulting microvesicles with subsequent functional effects on endothelial cells [80]. Furthermore, brain extracts from rats undergoing middle cerebral artery occlusion induced increased expression of miR-133b in MSC exosomes after co-culture [71].

4.3.5. Incubation

Interestingly, simple incubation of exosomes with the cargo of choice was also used as a method of loading exosomes. Curcumin was efficiently loaded into exosomes after only 5 min of incubation at 22 °C, and was shown to mediate significant anti-inflammatory effects in several disease models [90,92]. Curcumin is known to cause lipid rearrangement and changes in lipid fluidity of the cell membrane, which in the case of exosomes, may facilitate entry of the molecule into the lumen [99,100]. Both miR-150 and doxorubicin were loaded into exosomes by incubation at 37 °C for 1 and 2 h, respectively [81,88]. However, the production efficiency of encapsulated doxorubicin was much higher when loaded into exosome-mimetic nanovesicles derived from filtered cells [88,101]. The small size of the molecules loaded by simple incubation may enable passage across the exosome membrane, and be the reason for the efficiency of this method.

In addition to the choice of loading method, standardization of exosome purification methods could help to improve the reproducibility of the studies, and aid in the development of clinical scale production facilities. Thus, improvement of the currently available isolation protocols to increase the yield and purity of the resulting sample is of great value to the development of exosome-based delivery of cancer therapeutics, and a demand for quality assurance purposes in the clinic (for a thorough evaluation of the currently available isolation methods, please refer to Witwer et al. [3]). Since only few studies provide evidence for localization of the therapeutic cargo within the exosome lumen, new loading methods could be developed by taking advantage of possible interactions between the surface proteins and lipids with the therapeutic cargo, resulting in a complex formation that might be as efficient as luminal carriage.

4.4. Targeting peptides on the surface of the exosomes adds specificity to the action of the therapeutic cargo

The use of a targeting peptide or protein is a requirement for targeted therapies, and thus must be present on the surfaces of exosomes for these to be relevant drug delivery vehicles, especially for applications in cancer treatment if chemotherapeutics with major adverse side effects are to be delivered. At the moment, a substantial amount of papers has been published with regard to using exosomes in drug delivery, but only a small portion of them utilize a targeting strategy for direct delivery of the therapeutic cargo (Table 3).

Two studies bioengineered the lysosomal-associated membrane protein 2b (Lamp2b) to express a targeting peptide immediately below the signal peptide sequence, hence, securing the correct insertion of the protein into the exosome membrane, while avoiding cleavage of important regions in the targeting peptide sequence. Using this approach, the targeting peptides RVG and iRGD were successfully inserted into exosomes from immature dendritic cells to target either brain or tumor tissues [69,70]. Both studies found that usage of a targeting peptide on a Lamp2b pedestal significantly increased the specificity of

the treatment, and also enhanced the cellular uptake of the exosomes in the tissue of interest [69,70]. Ohno et al. generated a rather sophisticated fusion protein containing hemagglutinin, myc-tag, and a targeting peptide (GE11 or EGF) on top of the platelet-derived growth factor receptor (PDGFR). Using PDGFR as a pedestal allowed for expression of these components on the surface of the resulting exosomes. GE11 was shown to be an appropriate targeting peptide for drug delivery to tumor tissue, as it specifically bound the EGFR without eliciting any activation of the receptor [61]. Since EGFR is upregulated in cancer cells of several types of solid tumors, this type of targeting should be appropriate in overall cancer treatment [102].

Exploitation of a targeting peptide or protein on the exosome can be achieved by inducing overexpression of a particular protein in the exosome donor cell. Murine melanoma cells transfected with the CIITA gene to induce overexpression of MHC-II were shown to produce exosomes with enrichment of the MHC-II protein on the surface. These exosomes were targeted towards T-cells to elicit a potent Th1 response against cancer cells. Hence, the MHC-II molecule became both the targeting peptide and the therapeutic cargo in this model [67]. T-cell targeting was also achieved by using an antibody light chain surface coat on the exosomes [81]. Overexpression of the folate receptor α (FRα) on exosomes isolated from choroid plexus epithelial cells unexpectedly disclosed a mechanism by which the overexpression of FRα allowed for a specific entrance into the brain parenchyma through the choroid plexus [103]. Together with the results from another study, the use of exosomes may facilitate drug delivery to the brain by crossing the blood–brain barrier (BBB) or the choroid plexus to deliver therapeutics in malignant or neurodegenerative disorders, which remains a major obstacle for other types of drug delivery systems [69,103,104].

Exosomes or exosome-mimetic nanovesicles derived from U937 and Raw264.7 cells exhibited high level of efficiency in targeting chemotherapeutics to the tumor endothelial cells [88]. This resulted in a significant reduction in tumor volume, which the authors hypothesized (at least for the exosome-mimetic nanovesicles) as being due to the presence of LFA-1 from the donor cell membrane on the particle surface. LFA-1 would indeed facilitate binding to endothelial cell adhesion molecules (CAMs), and thus be targeted to rapidly growing tumors with extensive neovascularization [88]. As exosomes derived from tumor cells are known to possess specific characteristics compared to those secreted from non-malignant cells, this could be used as an advantage to increase the efficacy of exosome-based drug delivery to tumor tissues (see Section 1.3). Rana et al. showed that a web of the tetraspanin proteins on the surface of exosomes reflected both the origin and the target cell selection of exosomes. This knowledge could be utilized in directing exosomes towards specific tissues, hereby exploiting the normal physiology of the exosomes to facilitate targeted delivery into tumor tissues without the need of extensive bioengineering [98]. Future investigations should shed light on this interesting perspective. Another way to avoid the laborious process of having a modified membrane protein inserted into the exosomes might be to adsorb antibodies onto the exosome surface resulting in specific targeting combined with the endogenous characteristics of the exosome particles, e.g. the presence of fusogenic membrane proteins to facilitate cellular uptake.

4.5. Exosomes can be administrated via different routes to reach the area of disease

In order to possess any relevance in a clinical setting, exosomes must be stable and capable of delivering their cargo through the normally (preferably non-invasive) used administration routes. Such routes would be favorable to reach concealed tumors located in fragile tissues. Several administration routes have now been tested, which have generated interesting and encouraging results. Combined with the fact that exosomes are stable for long-term storage, exosome-based drug delivery seems appropriate in a clinical context [105,106]. The following sections summarize the administration routes used for exosome drug delivery in the studies published to date (see also Table 4).

4.5.1. Intravenous injection

The intravenous injection paradigm of exosomes is the most widely used route of administration across all the studies investigating exosome-based drug delivery (Table 4). Due to their endogenous origin, exosomes should principally avoid the removal by immune cells or avoid hepatic clearance to the same extent as exogenous nanoparticles. The latter is emphasized by the high abundance of exosomes in various body fluids [97]. Exosomes injected intravenously were delivered to several tissues including brain, pancreas, and especially tumor tissues [59,61,69,70,88,98]. The presence of leaky blood vessels and the lack of a proper lymphatic drainage (aka the enhanced permeation and retention effect) in solid tumors may favor extravasation and retention of the exosomes inside the tumor, as seen with other types of nanocarriers [2,107,108]. Intravenous injection is therefore an appropriate method for administration of exosomes, especially in malignancies.

The pharmacokinetic profile of exosomes in the circulation after intravenous injection was recently evaluated, showing that their half-life in the circulation is around 2 min with only a minimal presence after 4 h [66]. The exosomes were found to accumulate in the liver and later in the lungs, which suggests that the clearance of exosomes from the circulation is comparable to that of synthetic liposomes [66,109,110]. Distribution to lungs, liver, bone marrow and spleen of intravenously injected exosomes was observed in a study utilizing highly metastatic B16-F10 melanoma cells as donors. This distribution was thought to be due to the fact that these locations are organotropic sites of B16-F10 metastasis, which may be reflected on the released exosomes. The exosomes could still program bone marrow-derived MSCs to support tumor vasculogenesis, even though none of the exosome particles were present in the circulation after 24 h [47]. Accumulation in the liver was observed after administration of EGFR-targeted exosomes in a xenograft model of breast cancer, which might in part be caused by the high expression of this receptor in the hepatic tissue, in addition to tumor tissues [61,111]. Additional studies on the pharmacokinetics of exosomes from other cell types, including evaluations of distribution, degradation, and excretion are still warranted. It may also require

Table 3
The use of targeting peptides on the surface of exosomes.

Study	Targeting peptide	Target	Disease model
Tian et al. [70]	iRGD	Integrin αvβ3	Breast cancer
Jang et al. (exosomes and exosome-like nanovesicles) [88]	LFA-1	Endothelial cell adhesion molecules	Colon adenocarcinoma
Grapp et al. [103]	Folate receptor α	Not determined, but shown to target the brain parenchyma	None
Bryniarski et al. (exosome-like nanovesicles) [81]	Antibody light chain	Effector T cells	Allergic cutaneous contact sensitivity
Rana et al. [98]	Tetraspanins	Depends on the cell type	None
Ohno et al. [61]	GE11 or EGF	EGFR	Breast cancer
Maguire et al. [58]	BAP-TM	Biotinylated ligands	None
Lee et al. [67]	MHC-II	T-cells	Melanoma
Alvarez-Erviti et al. [69]	RVG	Acetylcholine receptor	None

additional modifications to the exosomes to avoid rapid clearance after intravenous injection. Such modifications could include PEGylation of the exosome particles, which is known to prolong the half-life of liposomes in the circulation [112].

4.5.2. Intratumoral injection

In cancer types, where the tumor is reachable without major invasive procedures, intratumoral injection of the exosome-encapsulated therapeutics is an appropriate administration route. Intratumoral injection was performed in three studies, which have resulted in successful tumor volume reduction after delivery of the exosome-encapsulated therapeutic cargo to the tumor cells [60,63,73]. This approach has advantages, because the direct injection secures specific delivery of the therapeutics. With regard to diseases of the brain, it may be favorable to avoid any invasive procedures that could damage this sensitive tissue. However, intratumoral injection, as seen in a study of GBM, may still have great potential, because tumor resection is the frontline treatment for GBM patients, and could possibly be combined with intratumoral administration of exosomes to reduce the risk of tumor recurrence [73,113].

4.5.3. Other types of administration routes

In addition to the administration routes described above, several studies have investigated the potential of other types of administration. Exosome-like nanovesicles extracted from grapes, administrated through the oral route, were shown to be stable when passaging through the gut, induce intestinal stem cell proliferation, and facilitate resolution of colitis [114]. This administration route was also successfully applied in another study, although intraperitoneal administration was superior to the oral route [81]. Intraperitoneal administration of loaded exosomes also facilitated investigation of the bioavailability of the anti-inflammatory substance, curcumin, showing that exosome-encapsulation increased stability of this compound in the circulation [92]. Subcutaneous administration of MHC-II-overexpressing exosomes facilitated immunization against murine melanoma, while injection into the brain ventricles and intranasal administration enabled entrance of the exosomes into the brain parenchyma [67,90,103]. The intranasally administrated exosomes also successfully delivered their therapeutic cargo to inhibit both inflammation and cancer of the brain [90].

The dosing of exosomes varies greatly between the studies. The use of very large doses in some studies compared to others (1–250 μg per injection in vivo, Supplementary Table 1) might be one of the reasons why therapeutic efficiency is still observed, even though the half-life of exosomes (in the systemic circulation) is rather short [66,69]. If large dosages of exosomes must be administrated in a clinical setting, it becomes immensely important to characterize the protein composition of exosomes. This would identify any potential immunogenicity in exosomes derived from different cells to avoid induction of adverse effects in the patient.

Table 4
Administration routes and dosages.

Study	Dose
Intravenous	
Tian et al. [70]	Not specified
Jang et al. [88]	10 μg total exosome protein
Bryniarski et al. [81]	30 μL resuspended exosome pellet
Takahashi et al. [66]	5 μg total exosome protein
Pan et al. [59]	200 μL 100-fold concentrated conditioned medium
Rana et al. [98]	250 μg total exosome protein
Ohno et al. [61]	1 μg total exosome protein
Alvarez-Erviti et al. [69]	150 μg total exosome protein
Oral	
Bryniarski et al. [81]	30 μL resuspended exosome pellet
Ju et al. [114]	1 mg total exosome protein
Intratumoral	
Mizrak et al. [63]	1 μL resuspended exosome pellet
Katakowski et al. [73]	50 μg total exosome protein
Kosaka et al. [60]	500 μL conditioned medium
Intraperitoneal	
Bryniarski et al. [81]	30 μL resuspended exosome pellet
Sun et al. [92]	4 mg/kg curcumin + exosomes
Intraventricular	
Grapp et al. [103]	10 μL resuspended exosome pellet
Subcutaneous	
Lee et al. [67]	20 μg total exosome protein
Intranasal	
Zhuang et al. [90]	2–10 μg total exosome protein or 12.5 pmol–1.5 nmol

5. Exosomes can serve as a blueprint for the design of new liposomal nanocarriers

One of the most studied types of drug carriers used in cancer therapy is the liposome. Liposomes are synthetic vesicles with a lipid bilayer membrane, which enables encapsulation of both hydrophobic and hydrophilic therapeutics. This results in increased stability and longer half-life of the drug, often together with fewer side effects [78]. For example, liposomal-encapsulated doxorubicin showed increased accumulation in tumor tissues, while the cardiotoxicity, normally seen with free doxorubicin, was significantly reduced [115]. Liposomes are normally produced in the same size range as that of endogenous exosomes to avoid uptake by the reticuloendothelial system (RES), and their uptake is mediated through pathways normally used by exosomes, such as endocytosis [115,116]. Thus, liposome-based drug delivery systems include components reflecting the endogenous behavior of exosomes.

A major difference between the synthetic liposomes and exosomes is the complex surface composition, which in the exosomes is characterized by an array of membrane proteins and special lipids (Fig. 3) [117]. The combination of this complex lipid composition and specific membrane proteins contributes to the efficient fusion between an exosome and its recipient cell. This uptake efficiency could not be mirrored when producing liposomes with the same lipid composition, but lacking the exosome membrane proteins [117,118]. As the web of tetraspanin proteins in the membrane of exosomes reflects the origin and target cell of an exosome particle, exploitation of these proteins may result in better targeting with regard to both exosome and liposome-mediated drug delivery to tumor tissues [98].

In theory, the half-life of exosomes in the circulation should be greater than that of liposomes due to their endogenous origin and special surface composition. Only few studies have investigated this thoroughly, and while some report that injected exosomes only accumulated in the liver to a small degree, other studies provide pharmacokinetic evidence of a circulatory half-life comparable to that of synthetic liposomes, partly due to liver accumulation [66,69,90].

One must take into account that the amount of evidence on liposomal drug delivery in cancer treatment with regard to therapeutic effect, production efficiency, and reproducibility of liposomal preparations is much higher than that of the current exosome standards, and the field of exosome-based drug delivery still lacks substantial experimental validation in vivo to elucidate its potential superiority to liposome or polymer-based nanocarriers. An advantage of liposome-based nanocarriers is the possibility to chemically modify the vesicle, facilitating release of the therapeutic cargo under specific microenvironmental conditions (e.g. stimulated by changes in pH or increased levels of matrix metalloproteinases in tumor tissues), which cannot be achieved with exosome-based drug delivery [108]. To address these issues, the exosome could be used as the blueprint for designing efficient nanocarriers, so that knowledge of the great complexity of exosome surface composition can be exploited to achieve better targeting and cellular uptake of future drug-delivering nanocarriers [119].

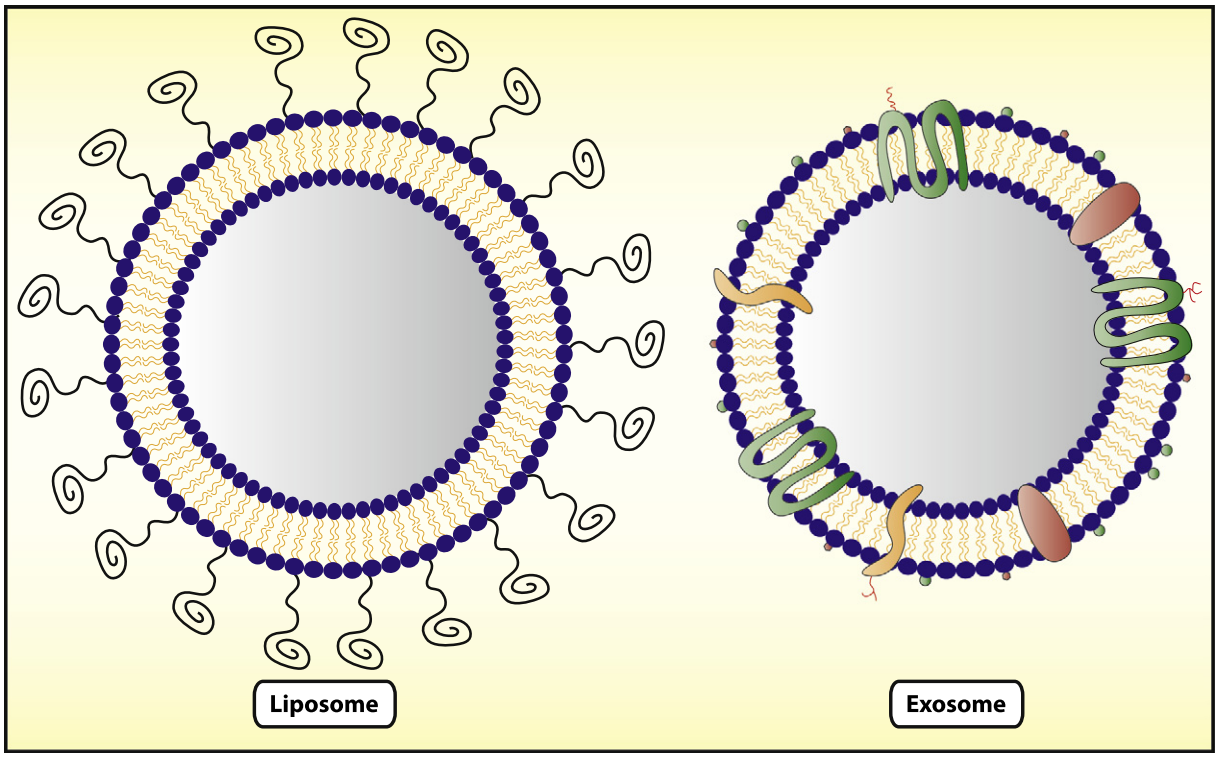


Fig. 3. Schematic representation of the liposome and exosome structure. Liposomes and exosomes are lipid bilayer structures exhibiting several individual differences with regard to their composition. Liposomes can be synthesized from different combinations of lipids, and their half-life in the circulation is often prolonged by the addition of polyethylene glycol (black curls). A major difference between the liposomes and exosomes is the complex surface composition, which in the exosomes is characterized by an array of proteins present in the membrane (such as tetraspanins (green)), and special lipids (green circles and red polygons) to facilitate efficient targeting and cellular uptake.

6. Exosome-based cancer therapies in clinical development

The research on exosome-based therapies is not limited to model disease systems. Several clinical trials are now either on-going or completed. In a phase I study, autologous dendritic cell-derived exosomes (Dex) were directly loaded with MAGE3 antigenic peptides and used as vaccination therapy for stage III/IV (metastatic) melanoma patients. Four intradermal and subcutaneous Dex vaccinations resulted in a significant increase in NK cell number and reconstitution of NKG2D expression in NK and CD8T cells. No toxicity was observed, and feasibility of autologous exosome production was established with Good Manufacturing Practice [120]. Dex-based vaccination loaded with MAGE antigens was tested against non-small cell lung cancer (NSCLC) in another phase I study, where the same NK cell reactivity was shown together with only minor side effects. Follow-up evaluations showed that time-to-progression ranged between 30 and 429+ days, while patient survival ranged between 52 and 665+ days [121]. In 2009, a phase II study was initiated to investigate second generation Dex (Dex2) in combination with chemotherapy in the treatment of unresectable NSCLC. Here, patients were treated with metronomic cyclophosphamide and vaccinated with Dex2 to obtain a greater level of immunostimulatory effects on T cells compared to first generation Dex, leading to sustained arrest of the disease (NCT01159288). Autologous ascites-derived exosomes (Aex) were tested in combination with GM-CSF in the treatment of colorectal cancer. This combination therapy showed a favorable cytotoxic T cell response directed against the tumor, a response not seen in patients treated with Aex alone [122].

The interesting effects of exosome-based vaccinations against different cancer types described above were further corroborated in a recently completed phase I clinical trial on malignant glioma. During tumor resection, glioma cells were isolated and treated with an antisense molecule drug to inhibit the expression of insulin-like growth factor receptor 1. Within 24 h, the resulting cells were re-implanted into the abdomen of the patients in small biodiffusion chambers. The lack of the specific surface receptor induces apoptosis in the re-implanted tumor cells, which causes a release of exosomes that are able to diffuse out of the chamber, the hypothesis behind this being that these exosomes will stimulate the immune system to induce a T cell-mediated antitumor response. The results of this trial are anticipated to be of great interest (NCT01550523).

Two clinical trials investigating plant-derived exosomes as cancer treatment are currently being conducted at the *James Graham Brown Cancer Center*. In the first trial, grape-derived exosome-like nanoparticles are being tested for their effects on oral mucositis and related pain after radio- and chemotherapeutic treatment of head and neck cancers (NCT01668849). Of particular interest with regard to drug delivery applications of exosomes in cancer treatment is the second clinical trial conducted at this research center. In this study, plant-derived exosomes loaded with curcumin are being evaluated for their efficacy in the treatment of colorectal cancer after oral administration (NCT01294072). All of these interesting studies (both on-going and completed), and the fact that exosome-based therapies exhibit proper safety profiles in a clinical setting, underscore the relevance of continuing the development of exosome-based drug delivery systems.

7. Concluding remarks

The field of exosome-based drug delivery has expanded greatly, and our understanding of possible applications has advanced within the last few years. Studies have highlighted eloquently many conditions in which exosomes can function as therapeutic carriers. In particular, it seems that cancer is well suited for exosome-based drug delivery, reflected in the many studies pertaining to cancer, and the extensive mapping of gene dysregulations that can be resolved after exosome-based delivery of interfering RNA. The emerging evidence that tumor cell-derived exosomes possess special characteristics may be used to

create an exosome-based drug delivery system superior to synthetic drug carriers. However, some shortcomings and obstacles do exist that need to be overcome to reach maximum potential in the clinic. Important issues that still need to be addressed include the choice of exosome donor cell (i.e. does it need to be autologous or can non-immunogenic exosome factories be generated?), type of loading procedure (i.e. which type of loading is most appropriate for specific types of cargo?), and use of targeting peptides on the exosome surface (i.e. can surface modifications like the ones published to date be suitable for clinical use?) (Fig. 2). The solutions to these questions are awaited with great interest. Further modifications to the surface of exosomes are warranted to increase the stability of intravenously injected exosomes, while choosing administration routes other than intravenous injection can be done depending on how easily the tumor tissues can be reached [66]. Rather encouragingly, several clinical trials on exosome-based therapies for cancer treatment are being conducted, some of which use exosomes as drug delivery vehicles [120,121]. This suggests that the clinical applicability of exosome-based therapies may be relevant not only for a large number cancer types, but also for other types of diseases in general. Finally, translation of the knowledge of exosome surface structure and physiology into generating synthetic exosome mimetics could be an interesting concept to pursue in order to obtain clinical scale production of nanocarriers with the same characteristics as exosomes [119].

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