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Review

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Exosomes as Drug Carriers for Cancer Therapy

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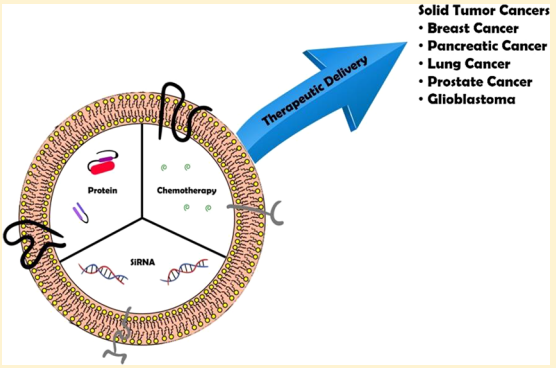
ABSTRACT: Exosomes, biological extracellular vesicles, have recently begun to find use in targeted drug delivery in solid tumor research. Ranging from 30−120 nm in size, exosomes are secreted from cells and isolated from bodily fluids. Exosomes provide a unique material platform due to their characteristics, including physical properties such as stability, biocompatibility, permeability, low toxicity, and low immunogenicity—all critical to the success of any nanoparticle drug delivery system. In addition to traditional chemotherapeutics, natural products and RNA have been encapsulated for the treatment of breast, pancreatic, lung, prostate cancers, and glioblastoma. This review discusses current research on exosomes for drug delivery to solid tumors.



KEYWORDS: *exosomes, solid tumor, drug delivery, nanoparticles*

1. INTRODUCTION

As a result of late diagnosis and limited treatment options, many malignant solid tumors have a poor prognosis and require more innovative approaches to cure such deadly disease. Targeted nanocarriers for drug delivery is a very promising avenue for treatment of solid cancerous tumors but have thus far been proven insufficient. With high clearance rates, toxicity to normal tissues, limited loading capacity, and shallow penetration depths, nanoparticles have proven difficult to use.[1,2] The common drug carriers, such as micelles, polymersomes, and liposomes, have failed to address these issues adequately.[1,2]

Although survival rates have increased in recent years, current treatments for many cancers remain ineffective and require the development of improved delivery methods. Among the cancers of breast, pancreas, lungs, prostate, and brain, the five-year survival rate is less than 22%, with glioblastoma being drastically lower (1%).[3,4] Solid tumor cancers are difficult to treat in all stages due to their unique characteristics in cell cycle and vasculature, which limit the delivery of drugs.[5,6] Current treatment methods include chemotherapy, radiation, and surgical resection when possible. Some natural products, such as curcumin, and anthocyanidins are currently either in the experimental stages or in clinical trials as adjuvant therapy.[7−13] However, the common chemotherapeutic drugs such as paclitaxel and doxorubicin suffer from low aqueous solubility and off-site toxic side effects, and as a result, new methods for targeted drug delivery are desired.[14,15]

Many clinical trials and research in recent years have utilized nanoparticles, specifically polymersomes, liposomes, and micelles as drug carriers. Exosomes are cell-derived nanoparticles with more advantages over these nanocarriers.[1] Exosomes are extracellular vesicles secreted by cells into bodily fluids, ranging in size from 30 to 120 nm, and carry a variety of biomacromolecules such as, RNA, DNA, proteins, etc.[16] The Minimal Information for Studies of Extracellular Vesicles 2018 guidelines state that the isolated extracellular exosomes must be characterized by at least three positive protein markers, including at least one transmembrane/lipid-bound protein or cytosolic protein as well as at least one negative protein marker.[17] In addition to the biological cargo, there are surface proteins and lipids that specify exosome origin and destination (Figure 1). These biomarkers are indicative of the cell type secreting the exosomes. The ability to differentiate surface proteins on the exosomes renders them as tools for early diagnosis of diseases.[18] Surface proteins may also be used in targeting and decreasing clearance rates, both features that polymersomes and liposomes frequently lack.[19]

Mediating cell−cell signaling, transportation of bioactive molecules, and assisting in immune response are some of the exosomes' known functions.[16] Inherent stability, biocompatibility, biological barrier permeability, low toxicity, and low immunogenicity are critical for the natural function of these lipid-based vesicles.[16] These characteristics address issues associated with other nanoparticle delivery vehicles, such as toxicity and high rate of clearance.[20] Unmodified exosomes can

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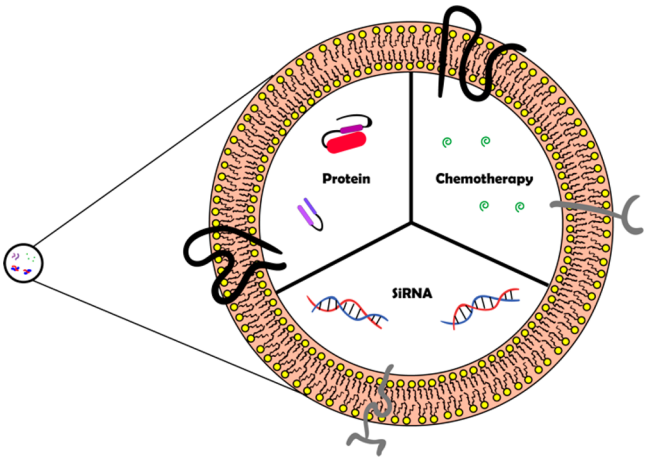

Figure 1. Schematic illustration of an exosome with the common cargos such as RNA and proteins.[24]

decrease proliferative effects in cancer cells.[20] When combined with different therapeutic strategies, exosomes decrease tumor proliferation with greater effectiveness.[21] In addition to carrying therapeutics, exosomes do not elicit an immune response in the bloodstream like other nanoparticle formulations.[21]

After the discovery in 1983, exosome research has steadily gained interest and momentum.[22] This review summarizes exosomal delivery of synthetic drugs, silencing RNA, microRNA, and natural products for solid tumors (summarized in Table 1). The manufacture, isolation, purification of exosomes, and drug loading are described in a recent review article.[23]

2. DISCUSSION

2.1. Exosomes for Drug Delivery to Breast Cancer.

Breast cancer is the most prevalent form of malignancy and the leading cause of cancer-related death in women in the western world.[11,12] Despite the decreased mortality rate with advancements in early detection and improvements to systematic adjuvant therapies, recurrence is seen up to 20 years after surgical interventions with increased metastasis and drug resistance.[27] Currently, the treatment options for breast cancer patients are surgical resection, chemotherapy, radiotherapy, and hormone therapy.[26] All current treatments have challenges, including drug resistance and toxicity to healthy tissues.[26] Targeted, nontoxic, and nonimmunogenic delivery technologies are needed to overcome the current challenges.[28] Exosomes have gained momentum as a potential targeted delivery vehicle for breast cancer.

Doxorubicin, a widely used chemotherapeutic drug for breast cancer, reduces the risk of recurrence up to 8% and mortality by 6.5%.[29,30] For patients taking doxorubicin, the side effects of congestive heart failure and drug resistance require a shift to less-effective therapy options.[29] Tian et al. loaded exosomes with doxorubicin for targeted delivery to triple-negative (MDA-MB-231) and estrogen receptor positive (MCF-7) human breast cancer cells.[28] Exosomes were isolated from mouse immature dendritic cells (imDCs) to minimize immunogenicity and further modified to express tumor targeting motif on the surface (iRGD) to maximize specificity. These cells were engineered to create exosomes that express lysosome-associated membrane glycoprotein 2b (Lamp2b) on the membrane. The Lamp2b was fused to the tumor-penetrating iRGD peptide to target the αv integrin, critical for the proliferation, migration, survival, and invasion of cancer cells.[28] Functionalized exosomes were loaded with doxorubicin using electroporation.[28] In an *in vivo* study, the drug-encapsulated, iRGD functionalized exosomes improved the effects of doxorubicin with no observable toxicity.[28] Hadla and colleagues also demonstrated that doxorubicin-encapsulated exosomes decreased cardiac toxicity and adverse effects on other tissues compared to the free drug.[17,18] Thus, the dose of doxorubicin can be increased, leading to a targeted cytotoxic effect on the breast cancer cells.[31,32]

Exosomes' natural ability to carry biologically relevant molecules is the main advantage over other nanoparticles. This characteristic has led to research in treatment options including the use of nucleic acid drugs[33−35] or activation of the immune system.[7,25] Current research in miRNA delivery is focused on breast cancer and other solid tumors. The miRNA-134, a tumor suppressant, is down-regulated in breast cancer.[34] O'Brien used exosomes to deliver miR-134 to Hs578Ts(i)$_8$ triple-negative breast cancer cells and observed that migration and invasion were reduced by 1.2-fold and the sensitivity to anti-Hsp90 drugs was enhanced by 2.1-fold.[34] Ohno and colleagues modified exosomes with the GE11 peptide, which specifically binds EGFR, and were loaded with let-7a miRNA, a regulator for the reduction of cell division and alteration of cell cycles. The epidermal growth factor receptor (EGFR)-expressing breast cancer cell lines (HCC70, HCC1954, and MCF-7) were used to test the exosomes' effectiveness.[35] The targeted and drug-loaded exosomes were delivered to EGFR-expressing xenograft breast cancer tissue in RAG2$^{-/-}$ mice.[35] These studies resulted in suppressed tumor growth (Figure 2)

Table 1. Overview of Cancer Type, Exosomal Cargo, and Source of Exosomes Discussed in This Review

cancer type	cargo	source of exosomes
breast	curcumin	TS/A, 4T.1 and B16 cells[7]
		raw bovine milk[9]
	anthocyanidins	raw bovine milk
	paclitaxel	macrophage cells[14]
		raw bovine milk[15]
	soluble proteins	HEK293, HT1080, and HeLa cells[19]
	Berry Anthos	raw bovine milk[21]
	doxorubicin	MDA-MB-231 and MCF7 cells[28]
	siRNA	HEK 293 and MCF7 cells[33,35]
	miRNA	HEK 293 and MCF7 cells[33,35]
	ssDNA	HEK293 and MCF7 cells[33]
	miR-134	Hs578T cells[34]
	trastuzumab	modified dendritic cells[25]
pancreatic	oncogenic Kras	human foreskin fibroblast cells[40]
		bone marrow-derived mesenchymal stem cells[43]
lung	celastrol	raw bovine milk[44]
	paclitaxel	macrophage cells[47]
	doxorubicin-gold nanoparticle conjugate	H1299 and YRC9 cells[48]
	peptide	peripheral blood mononuclear cells[49]
prostate	paclitaxel	LNCaP and PC3 cells[59]
glioblastoma	curcumin	GL26 cells[62]
	STAT3 inhibitor	Gl26 cells[62]
	rhodamine 123 with paclitaxel or doxorubicin	brain neuronal glioblastoma-astrocytoma U-87 MG, endothelial bEND.3, neuroectodermal tumor PFSK-1, and glioblastoma A-172 cells[66]
	MiR-124a	mesenchymal stem cells[73]
	siRNA	bEND.3 cells[66]

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and provided another promising strategy for the delivery of nucleic acid drugs.[35]

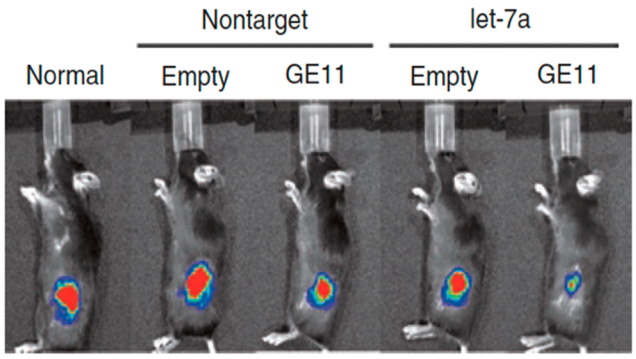

Figure 2. Human embryonic kidney cells (HEK293) expressing GE11 were transfected with synthetic let-7a. Exosomes containing let-7a were purified from culture supernatants and intravenously injected (1 μg of purified exosomes, once per week for 4 weeks) into mice bearing luciferase-expressing breast cancer cells HCC70. Reproduced with permission from ref 35. Copyright 2013 Elsevier.

Due to tumor avoidance of the immune system, activating immune response using an exosome-based vaccine is a promising strategy for cancer.[7] Patients treated with trastuzumab, a chemotherapeutic monoclonal antibody, commonly develop resistance, making a delivery method an urgent necessity.[25] Exosomes from dendritic cells were transfected with adenoviral vector (AdV_{HER2}), creating a vaccine.[25] The vaccine was used for treatment in mice with trastuzumab-resistant BT474 and trastuzumab-sensitive MCF-7 tumors.[25] The vaccine stimulated the cytotoxic T lymphocyte response and was observed to kill cancer cells and eradicate tumors, providing a promising new strategy for drug-resistant tumors.[25] Despite the *in vitro* and *in vivo* stages, the exosome delivery strategies are promising methods for new breast cancer therapy. The exosomes provide solutions to many challenges that are faced by clinicians in the treatment of breast cancer, such as off-site toxicity and drug resistance. Clinical trials of exosome-based delivery methods are expected to occur due to the positive published results.

2.2. Exosomes for Drug Delivery to Pancreatic Cancer. With a single-digit five-year survival rate, pancreatic cancer is deadly due to the inability to detect early and treat metastatic tumors.[36,37] Most conventional and targeted therapies fail to provide substantial response primarily due to the limited delivery efficacy of cytotoxic agents.[38] One way to combat this problem is to utilize a targeted nanosized drug delivery vehicle. Recently, the FDA approved a nanoparticle delivery strategy for the anticancer drug paclitaxel (Abraxane).[39] This monumental leap in the treatment of pancreatic cancer has accelerated the development of nanoparticle-based drug delivery methods. Abraxane, initially approved for metastatic breast and nonsmall cell lung cancers, utilizes albumin-bound paclitaxel.[36] Abraxane in conjunction with gemcitabine leads to increased effectiveness for pancreatic cancer patients, making this plan first line treatment.[36]

One of the biggest challenges of nanoparticle-mediated drug delivery is the high rate of clearance. Due to exosomes' natural characteristics, they have longer retention in the circulation compared polymersomes or liposomes.[40] The increased retention time results from a transmembrane protein (CD47-SIRPα), which prevents exosomes from being phagocytosed and therefore increase the delivery efficacy of its content to the target sites.[40] Increased retention time leads to higher concentrations of exosomal cargo to pancreatic cancer cells and enhanced treatment effectiveness.[40] In addition, exosomes also enhance macropinocytosis of cancer cells, one mechanism of uptake.[40] While Abraxane has made significant advances in the treatment of pancreatic cancer, it is still not enough, and exosomes have the potential to a better option.

Due to exosomes' ability to effectively carry macromolecules, a silencing RNA can be encapsulated, turning off specific genes within the cancer cells.[40] Kamerkar employed exosomes to carry a siRNA against the oncogenic protein Kras ($Kras^{G12D}$).[40,41] Oncogenic Kras is a signaling protein that drives the mutation of pancreatic cancer formation. Silencing oncogenic Kras using this approach showed unprecedented tumor regression and the potential to target pancreatic cancer.[40] In orthotopic and genetically engineered mouse model systems, siRNA encapsulated exosomes (iExosomes) showed superior antitumor efficacy (Figure 3), with decreased pancreas desmoplasia.[40] Antitumoral effect of iExosomes was accompanied by enhanced cancer cell apoptosis, suppressed proliferation, and reduced phospho-ERK, phospho-AKT, and oncogenic Kras levels *in vivo* experiments.[40] The engineered Kras encapsulated exosomes showed decreased clearance rates compared to plain exosomes.[40] Exosome research in pancreatic cancer is a rapidly advancing field as targeted drug delivery is the most viable solution to the treatment of pancreatic cancer.[21] siRNA is limited in use by the challenges of delivery to target organs during clinical trials.[42] The body's normal

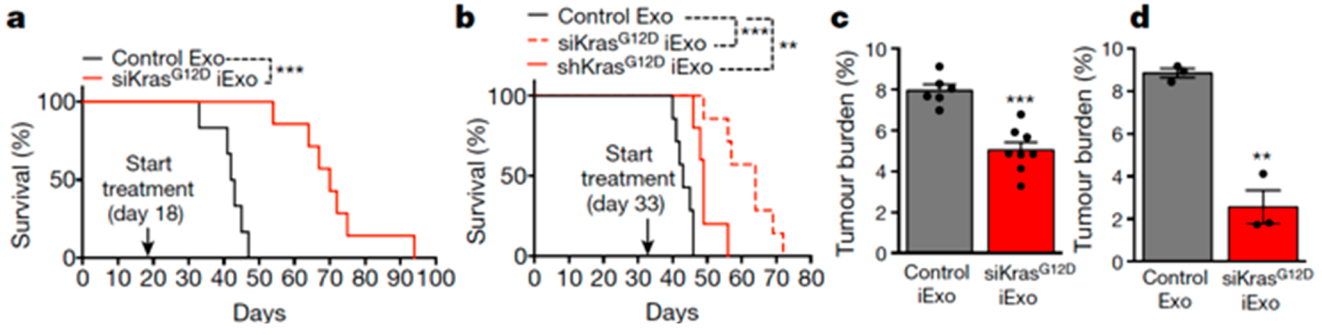

Figure 3. Kras iExsomes suppress pancreatic cancer progression in genetically engineered mouse models for pancreatic cancer. Kaplan−Meier survival curve of tumor-bearing mice with early (a) or late (b) treatment of iExosomes. (c) Tumor burden (early treatment) at the experimental end point. (d) Tumor burden at 44 days of age. Reproduced with permission from ref 40. Copyright 2017 Springer.

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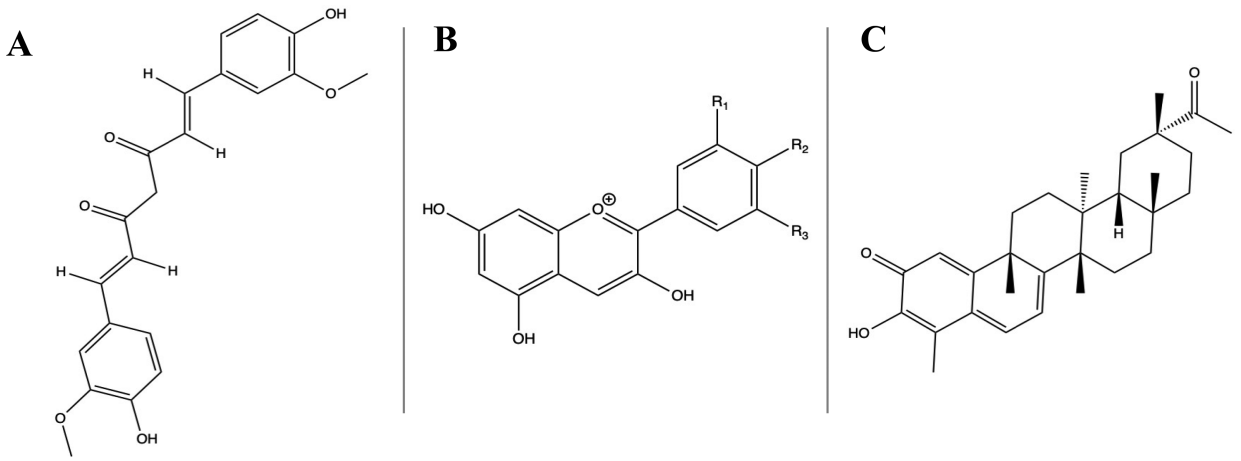

Figure 4. Chemical structures of natural substances used to treat lung cancer: (A) curcumin, (B) anthocyanidins, and (C) celastrol.

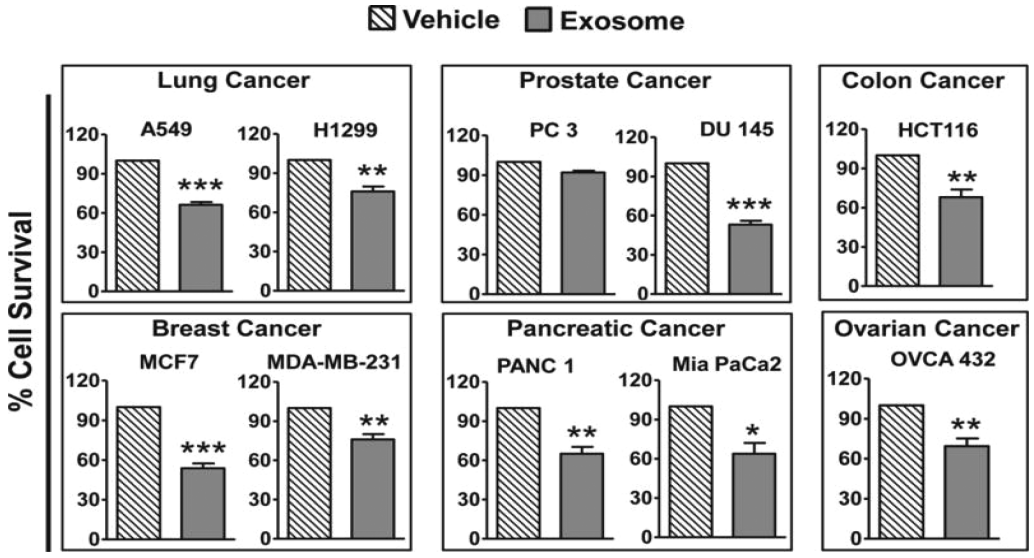

Figure 5. Exosomes (vehicle) and exosomes containing Anthos were tested for cytotoxic effects on various cancer cell lines. Cancer cell lines of lung, breast, ovarian, colon, pancreas, and prostate were treated with 50 μg/mL exosomes for 72 h, and effect on cell growth inhibition was assessed by MTT assay and compared with untreated cells. Statistical analysis was performed using Student t test to compare exosomes alone with vehicle treatment. *$p \leq 0.05$; **$p \leq 0.01$; and ***$p \leq 0.001$. Reproduced with permission from ref 11. Copyright 2017 Elsevier.

physiologic processes, such as renal filtration, breakdown by enzymes, and phagocytic cells have hampered the translation from *in vitro* to *in vivo* use.[42] Multiple research groups are optimizing the use of these small RNA molecules with successful early studies queuing interest from the field. The new ideas bring great promise to pancreatic cancer and the ability to decrease patient suffering while increasing effective treatment options.

Subsequently, Mendt et al. scaled up the production of exosomes.[43] They also demonstrated that the bone marrow-derived mesenchymal stem cell exosomes encapsulating siRNA (iExo) retain the efficacy for six months when stored frozen.[43] The research group tested the iExo on patient-derived xenograft mice model of pancreatic cancer with positive results.[43] *In vivo* studies demonstrated significant increases in life expectancies of mice treated with a combination of iExo and gemcitabine in both early and late stage diseases (mice surviving for 90 days in total).[43] In an early stage pancreatic tumor, mice had greater than 50% survival at day 89 when the study was terminated.[43] iExo are now making their way into Phase I clinical trials but have not begun recruiting patients yet. The new idea of iExo brings great promise to pancreatic cancer and the ability to decrease patient suffering while increasing effective treatment options.

2.3. Exosomes for Drug Delivery to Lung Cancer. Lung cancer accounts for one out of four cancer-related deaths, making it the leading cause of mortality worldwide.[44] However, current therapeutic interventions are not efficient, and most are palliative.[44] Researchers are investigating natural products or synthetic drugs for use as a chemotherapeutic. Despite the development of substances for preventing progression and inhibiting malignancy of lung cancer, clinicians have been struggling with successful targeted delivery. The use of naturally occurring compounds is desired for their cost-effectiveness and feasibility for oral administration. However, many suffer from bioavailability and toxicity issues, making their use difficult due to lack of delivery methods.[44] Exosomes have been explored as a potential delivery method to overcome bioavailability, toxicity, and clearance.[44]

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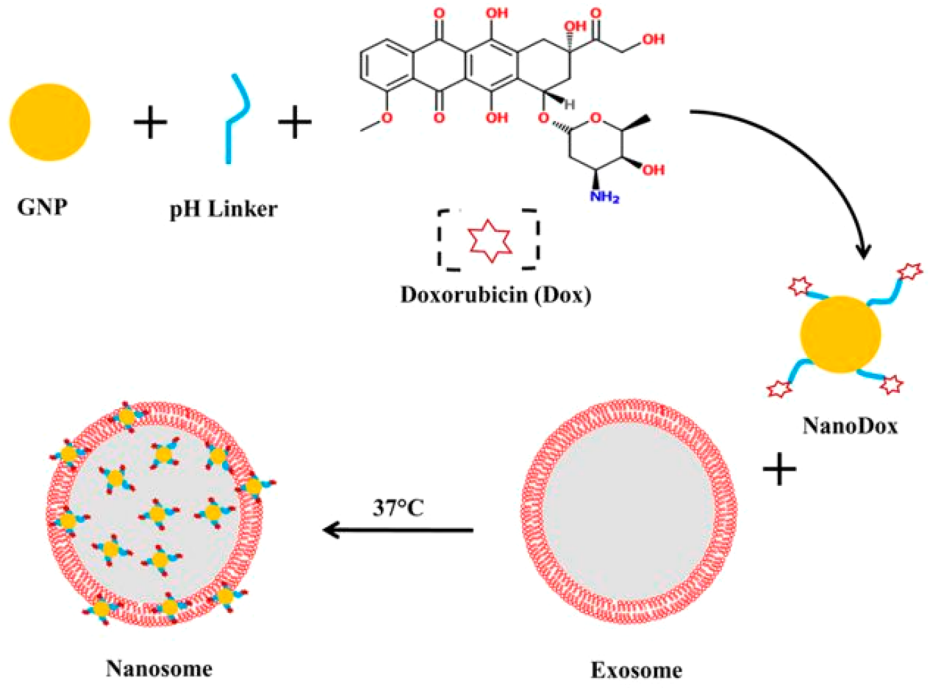

Figure 6. Schematic of nanosome synthesis with encapsulated doxorubicin. Reproduced with permission from ref 48. Copyright 2016 Springer.

Three different naturally occurring substances that have been explored as possible treatments of lung cancer are celastrol, curcumin, and anthocyanidins (Figure 4). Celastrol is a natural product (isolated from *Tripterygium wilfordii* and *Celastrus regelii*) shown to have antiproliferative and antitumor properties but has limited therapeutic use due to low bioavailability and off-site toxicity.[44,45] Aqil and colleagues studied the possibility of milk-derived exosomes delivering celastrol in an *in vitro* and *in vivo* lung cancer model.[44] In the *in vitro* assessment, human lung cancer H1299 cells were treated with free celastrol and celastrol-loaded exosomes.[44] The *in vivo* model used within this study was nude mice with a subcutaneous injection of H1299 lung cancer xenografts.[44] The antiproliferative effect of celastrol was further enhanced when encapsulated in exosomes in both the *in vitro* and *in vivo* setting.[44] The use of encapsulated celastrol in exosomes can reduce the toxicity while increasing the efficacy and has the potential to be a novel treatment of lung cancer.[44]

Curcumin (isolated from turmeric) has been studied extensively as a potential chemopreventative for cancer.[7−9] Curcumin has poor water solubility due to hydrophobicity, reducing its clinical efficacy.[46] To enhance delivery of the compound, bovine milk-derived exosomes were loaded with curcumin and tested in *in vitro* lung cancer models.[9] With the addition of curcumin-loaded exosomes, growth inhibition increased in the lung cancer cells without any toxic side effects to healthy cells.[9]

Aglycones (anthocyanidins) are naturally occurring substances found in berries possessing antiproliferative, apoptotic, anti-inflammatory, and antioxidant properties, but suffer from low permeability and oral bioavailability.[11] Munagala et al. developed a method for loading milk-derived exosomes with anthocyanidins for oral delivery to mice with lung cancer xenografts.[11] The group initially tested the novel delivery method of plain exosomes and exosomes containing anthocyanidins *in vitro* using A549 and H1299 human lung cancer cells and observed a 66% and 76% reduction in cell number, respectively (Figure 5).[11] When the exosomes were loaded with the anthocyanidins, up to a 30-fold decrease in cell survival was observed as compared to free compounds in lung cancer cell lines (Figure 4).[11] When tested *in vivo*, anthocyanidine-loaded exosomes also increased therapeutic response compared to free compounds without any toxicity.[11] The use of exosomes for the delivery of natural products provides a promising method to overcome the challenges of bioavailability and toxicity.

In addition to the natural products, researchers have explored encapsulating synthetic pharmaceuticals in the exosomes. Kim et al. developed another method for loading paclitaxel via sonication into exosomes released by macrophages.[14] Exosomes loaded with paclitaxel were shown to be a promising strategy for drug delivery to multidrug resistant pulmonary cancers.[14] In a later study, the group modified the exosomes with the aminoethylanisamide-polyethylene glycol (AA-PEG) vector for targeting the sigma receptor, a commonly overexpressed receptor in nonsmall cell lung cancer.[47] The exosomes were biocompatible, long-circulating, and targeted drug delivery vehicles with innate features of macrophages.[47] The targeted exosomes increased the survival of the mouse model while decreasing toxic side effects.[47] Agarwal et al. developed paclitaxel-loaded exosomes for use as an oral delivery method.[15] The exosomes were isolated from raw cow milk and loaded with paclitaxel by mixing.[15] The group found the orally administered paclitaxel-loaded exosomes decreased the toxicity and increased the therapeutic efficiency of the drug to A549 xenograft lung tumor in mice.[15]

Other groups are working on loading exosomes with doxorubicin for possible lung cancer therapy.[48] Srivastava et al. investigated the efficacy of exosomes encapsulating doxorubicin conjugated to gold nanoparticles (GNPs) as a drug carrier.[48] In a separate study, Srivastava et al. explored exosomes for delivery of doxorubicin-GNPs by a pH-sensitive hydrazine linker, which they called nanosomes (Figure 6).[48] The efficacy of the exosomes were evaluated in an *in vitro*

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setting using two nonsmall cell lung cancer cell lines, H1299 and A549, and one lung fibroblast cell line, MRC9.[48] The exosomes showed preferential cytotoxicity to lung cancer cells compared to healthy cells as evidenced by the reduced viability of the H1299 and A549 cells compared to MRC9 cells.[48]

In addition to the promising *in vitro* and *in vivo* results, Morse et al. conducted a Phase I clinical trial using exosomes encapsulating tumor antigens for advanced nonsmall cell lung cancer.[49] Exosomes derived from dendritic cells from the patient and loaded with MAGE tumor antigens were given four times weekly to study participants.[49] The therapy was well tolerated, and some of the participates experienced long-term disease stability.[49] To investigate further, Phase II clinical trials are planned to expand the number of patients.[49] The use of exosomes as a drug carrier for lung cancer shows considerable translational potential to overcome the current challenges faced by clinicians.

2.4. Exosomes for Drug Delivery to Prostate Cancer. Prostate cancer is the most frequently diagnosed malignancy in the United States, being the third most common type of cancer death in men.[50] Successful treatment of prostate cancer is difficult due to the rate of metastasis and late detection.[51,52] If treated early with surgical intervention, radiation, or hormone therapy, moderate success has been observed but can become devastating with tumor metastasis.[52] Specific biomarkers are required for determining the type of prostate cancer, and exosomal surface proteins can be used for early detection of the malignancy.[53−58]

In addition to early detection, exosomes show promise as a chemotherapeutic carrier by increasing the cytotoxic effect and toxicity of chemotherapeutic on cancer cells.[45] Saari et al. isolated exosomes from the conditioned culture media of LNCaP (androgen-sensitive human prostate adenocarcinoma) and PC-3 (prostate adenocarcinoma) cells using ultracentrifugation. Subsequently, the exosomes were loaded with paclitaxel, and assays showed decreased prostate cancer cell viability.[59] To determine the importance of exosomal surface proteins, all surface proteins were removed from paclitaxel-loaded exosomes.[59] Although there were no indications of formation problems, the delivery efficiency for paclitaxel decreased.[59] This is likely due to the decreased entry of the in the cancer cells. The surface proteins partially mediate endocytosis of the exosomes.[59] The surface proteins are vital in the drug delivery properties of exosomes due to their specific mechanisms of entering cells.[59]

In addition to chemotherapeutic drug delivery, exosomes have been researched as a vaccine for prostate cancer and a delivery method for the anti-inflammatory agent, curcumin (previously discussed in the lung cancer section of this article).[60−62] With these potential applications of exosomes for the treatment of prostate cancer, there is hope that the most frequently diagnosed cancer will begin to have higher survival rates in the near future.

2.5. Exosomes for Drug Delivery to Glioblastoma. Even with a multimodal treatment plan, often consisting of surgery, radiation, and chemotherapy, the median survival remains under 15 months for glioma.[63] The most prevalent form of glioma, tumors arising from glial precursor cells, is glioblastoma multiforme (GBM).[64] Frontline therapy for GBM is termed the Stupp protocol, involving concurrent radiotherapy along with Temozolomide-based chemotherapy.[65] Heterogeneity among glioblastoma leading to both inter- and intratumor variation results in altering responses to therapy, warranting novel therapeutic strategies. The blood−brain barrier (BBB) remains near impenetrable, blocking the penetration of more than 98% of all small molecule drugs.[66] Exosomes with endless variation in loading capacity and homing abilities showcase a possible treatment modality for GBM,[65] allowing for new and old classes of drugs to be effectively delivered to their target sites.

Yang et al. showcased the ability of drug-loaded exosomes to cross the BBB *in vivo*, using a zebrafish model.[66,67] The propensity of zebrafish as a reliable *in vivo* BBB model was shown by Jeong et al., by confirmation of specific characteristics seen in higher order vertebrates.[68] Exosomes were isolated from cell culture media from various cell lines including GBM U-87 MG, brain endothelial cells bEND.3, neuroectodermal tumor PFSK-1, and glioblastoma A-172. Paclitaxel and doxorubicin were incorporated into the exosomes using electroporation along with a fluorescent dye (rhodamine 123) and then tested for CD9, CD63, and CD81. No significant differences were found between cell lines except bEND.3 cells. These cells had a near 2000 times greater expression of CD63, possibly eluting to a unique receptor-mediated transport mechanism for crossing the BBB.[66] This theory was partially confirmed by incubating bEND.3 cells with rhodamine 123 exosomes. This resulted in a significantly higher cellular uptake, showing the involvement of active transport mechanisms. Exosome delivery across the zebrafish BBB was tested using all four cell types (Figure 7). While the exosomes from glioblastoma-astrocytoma (U87-MG), neuroectodermal tumor (PFSK-1), and glioblastoma (A-172) cells failed to cross the BBB, the bEND.3 exosomes were successful. The crossing of the BBB by drug encapsulated bEND.3 exosomes resulted in reduced tumor size compared to free drug and control treatments.

Yang et al. attempted to further their zebrafish studies by loading siRNA in the exosomes.[66] After finding the significant uptake by bEND.3 exosomes,[66] the group loaded VEGF siRNA into the isolated exosomes. siRNA alone was unable to cross the zebrafish BBB effectively. However, the bEND.3 siRNA loaded exosomes again showed utility (Figure 8).[66,67] The siRNA loaded exosome decreased the cellular fluorescence signal of the *in vivo* DiD-labeled cells by a factor of 4. This decrease in cellular fluorescence and supporting results from the paper indicated siRNA loaded exosomes could cross the blood−brain barrier while inhibiting VEGF in this xenographic mouse model.[67]

A second type of RNA related therapeutic, miRNAs also show anticancer characteristics by their ability to alter the posttranscriptional gene expression.[69−71] Expression levels of specific miRNAs have been implicated in GBM showing downregulation relative to non-neoplastic brain tissues.[72] Lang et al. screened eight miRNAs found to have implications in GBM against five glioma stem cell lines representing all GBM subtypes.[73] miRNA-124a was selected based on effectiveness in decreasing cellular viability across all five cell lines.

Using a lentivirus, the group was able to overexpress miRNA-124a in cultured mesenchymal stem cells (MSCs). Exosomes were harvested from MSC cultures that are transfected with the cDNA for miRNA-124a, a nonsense control cDNA (miRControl), or medium only (Exoempty). The exosomes were lysed, and RNA was collected for qRT-PCR analysis. Exosomes from miRNA-124a transfected MSCs had a 60 -fold increase vs miRControl or medium only exosomes.[73] Lang et al. last tested their exosomes *in vivo* using

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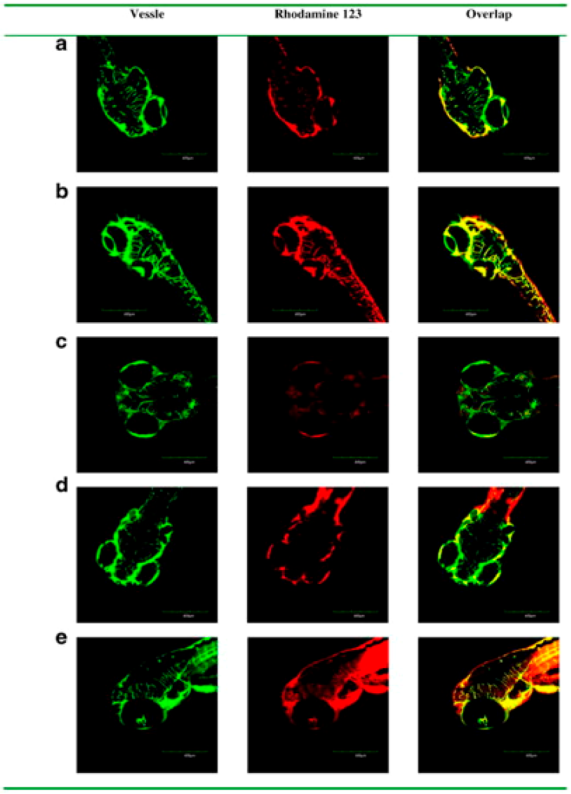

Figure 7. *In vivo* brain imaging of exosome delivered rhodamine 123 in Tg (fli1: GFP) embryonic zebrafish. Rhodamine 123 (red) retained within vessels (green) after the injected formulations without exosome (a) and with exosomes isolated from (b) neuroectodermal tumor PFSK-1, (c) glioblastoma A-172, and (d) glioblastoma-astrocytoma U-87 MG. Rhodamine 123 (red) dispersed out of vessels (green) after the injected formulation with exosomes isolated from (e) brain endothelial bEND.3 cells. Reproduced with permission from ref 66. Copyright 2015 Springer.

surgically implanted GBM cells in mice. Exo-miR control, and Exo-empty treated animals died within 48 days with a median survival of 41 days, while Exo-miRNA124 animals showed a median survival of 104 days (Figure 9).[73]

The BBB provides a necessary protection system; however, it also provides a barricade against treatment. Practically all large molecule pharmaceutics including monoclonal antibodies, recombinant proteins, and RNA-like molecules are unable to pass through the BBB on their own.[74] Exosomes appear as a viable option for a delivery vehicle capable of carrying both large and small molecules across the blood−brain barrier.

3. CHALLENGES

Despite the numerous advantages, there are several challenges of using exosomes as drug carriers, the major challenge being the composition of exosomes and their functions. Exosomes are involved in cellular communication through the transport of biomacromolecules from the host to the recipient cell. However, it is unclear exactly which molecules are transported, their roles, and the associated heterogeneity of the exosomes.[57] It is also necessary to choose appropriate donor cells to prevent triggering an immune response.[75] Even when the source cells are identified, the scale-up operation to produce a sufficient amount of the vesicles for effective treatment is another impending problem.[26] Exosomes isolated from large-scale cell cultures also suffer from the heterogeneity of the

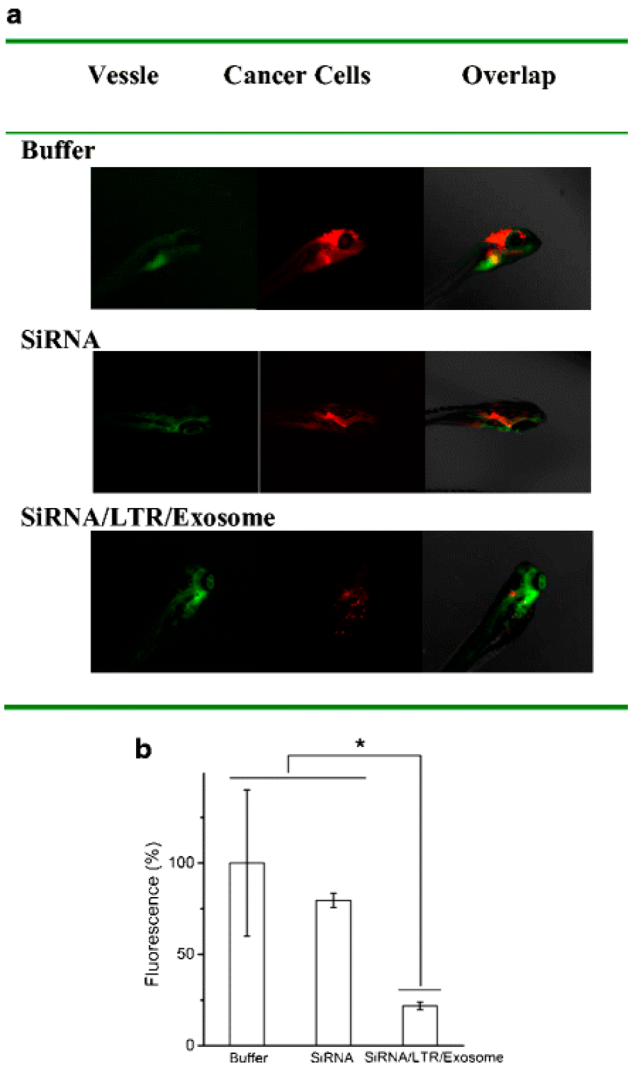

Figure 8. Efficacy of exosome-delivered VEGF siRNA in a zebrafish cancer model. Images (a) and statistical analysis (b) of quantified DiD-labeled (red) cancer cells in the zebrafish brain. *Results are significantly different ($p < 0.05$). Data represent the mean ± SD, n = 12(5). Reproduced with permission from ref 67. Copyright 2015 Springer.

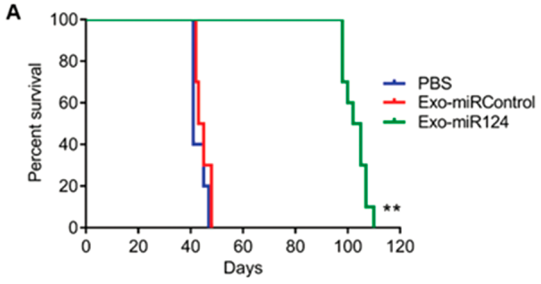

Figure 9. Percent survival of mice treated *ex vivo* with Exo-miR124 after being implanted with GSCs. **$P < 0.01$. Reproduced with permission from ref 73. Copyright 2018 Oxford University Press.

biomolecules on the surface and inside the aqueous lumen. In addition, exosomes from cancer cells can trigger metastasis of the disease.[58,76−81] Bovine milk exosomes may provide a solution to these challenges due to the ease of isolation, scale-up, and the lack of immunogenicity.[82,83] Clearly, further

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research is required into the composition of exosomes and their efficacy as a human drug carrier.

4. CONCLUSIONS

Exosomes are potential drug carriers in the early stages of development and validation.[56,57] Exosomes possess the ability to communicate to cells with distinct biomarkers.[16] Recent studies show their capabilities and indicate their potential as an effective drug carrier. Exosomes' ability to fight solid tumor cancers comes at a much needed time, with many current nanoparticle delivery systems failing at a rate of 90 ± 5% in industry and government settings.[1] Lung, pancreatic, glioblastoma, prostate, and breast cancers are deadly malignancies and require more specialized treatment methods. Current approaches do not treat many of these cancers successfully. The biological origin of the exosomes renders them with a unique ability to address current issues with nanoparticle-based drug delivery.[56,57] With decreased clearance and increased specificity due to surface proteins and engineered targeting methods, exosomes are very promising drug carriers to target and deliver chemotherapeutics, RNA, and natural products. However, the exosomes are not devoid of challenges either. The heterogeneity of the encapsulated and surface molecules, potential immunogenicity, and the risk of promoting metastasis need to be overcome before successful therapeutic use of the vesicles in human solid tumors. Clinical trials are still on the horizon for the use of exosomes as a drug carrier for solid tumors.

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The manuscript was written through the contributions of all authors.

Notes

The authors declare no competing financial interest.

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