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REVIEW PAPER

Exosomes as Tools to Suppress Primary Brain Tumor

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Abstract Exosomes are small microvesicles released by cells that efficiently transfer their molecular cargo to other cells, including tumor. Exosomes may pass the blood–brain barrier and have been demonstrated to deliver RNAs contained within to brain. As they are non-viable, the risk profile of exosomes is thought to be less than that of cellular therapies. Exosomes can be manufactured at scale in culture, and exosomes can be engineered to incorporate therapeutic miRNAs, siRNAs, or chemotherapeutic molecules. As natural biological delivery vehicles, interest in the use of exosomes as therapeutic delivery agents is growing. We previously demonstrated a novel treatment whereby mesenchymal stromal cells were employed to package tumor-suppressing miR-146b into exosomes, which were then used to reduce malignant glioma growth in rat. The use of exosomes to raise the immune system against tumor is also drawing interest. Exosomes from dendritic cells which are antigen-presenting, and have been used for treatment of brain tumor may be divided into three categories: (1) exosomes for immunomodulation-based therapy, (2) exosomes as delivery vehicles for anti-tumor nucleotides, and (3) exosomes as drug delivery vehicles. Here, we will provide an overview of these three applications of exosomes to treat brain tumor, and examine their prospects on the long road to clinical use.

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Introduction

The use of exosomes as therapeutic delivery agents is a novel biological non-cell-based treatment strategy. Exosomes pass the blood–brain barrier, and can be used to deliver small biological or pharmaceutical molecules to tumor in brain. A small, but growing contingent of researchers are developing the use of exosomes as delivery vehicles for the treatment of primary brain tumor. Due to technical difficulties, and multiple methods of isolation and classification, there is currently no consensus about the nomenclature of cell-derived vesicles which includes exosomes. In the following, we employ the term ‘exosome’ to refer to extracellular vesicles of 50–150 nm in diameter that carry RNA, which includes exosomes, but does not exclude other microvesicles of a similar size. Here, we discuss the current state of this emerging field, and consider opportunities and obstacles that lay on the road to clinical application.

Long considered to be cellular waste products, exosomes are now also recognized as intercellular signaling vesicles that coordinate short and long-range cellular communication transfer by shuttling proteins and nucleotides between cells (Thery 2011). Exosomes are actively taken up by recipient cells, whereby cellular processes can be altered (Hu et al. 2012). Exosome uptake appears to depend on the type of acceptor cells, and it occurs primarily through phagocytosis or endocytosis (Christianson et al. 2013; Cocucci and Meldolesi 2015; Feng et al. 2010). It appears that most, if not all, cells produce exosomes. Exosomes contain proteins, lipids, and miRNA capable of

regulating a variety of target genes by transcriptional and post-transcriptional mechanisms (Mathivanan et al. 2010; Miller and Grunewald 2015). Of note, extracellular RNAs may also be contained in other extracellular vesicles, or associated with proteins, and high- or low-density lipoproteins (Arroyo et al. 2011; Vickers et al. 2011). As natural vectors for bio-molecular transfer, there is growing interest in using exosomes as therapeutic delivery vehicles.

Early interest in exosomes in tumor focused upon their potential as diagnostic markers. The cargo of exosomes reflects the genotype and phenotype of their parent cells and their nucleic acid and protein cargo is preserved from degradation, thus the notion that 'liquid biopsies' might reveal useful prognostic information is an attractive one, particularly in cancer (Keller et al. 2011; Li et al. 2009; Raposo and Stoorvogel 2013; Santiago-Dieppa et al. 2014; Skog et al. 2008; Tavoosidana et al. 2011). Glioblastoma exosomes escape the blood–brain barrier, with likely systemic and distal signaling and immune consequences (Graner et al. 2009; Skog et al. 2008). To date, definitive evidence for the diagnostic or prognostic value of exosomes as biomarkers remains elusive; however, technical hurdles involving exosome isolation and cargo analysis are as likely to be blamed as biological complexity and variation, and rapid progress is being made (Miller and Grunewald 2015; Shao et al. 2012; Tickner et al. 2014). For example, Shao et al. recently demonstrated a miniaturized nuclear magnetic resonance system that rapidly detected glioblastoma-shed microvesicles in clinical blood samples with a sensitivity that exceeded standard ELISA and flow cytometry assays (Shao et al. 2012).

The idea of using exosomes as therapeutic agents has existed for more than a decade, and has been gaining interest. As endogenous mediators of intercellular gene-regulation, exosomes represent efficient carriers that can deliver therapeutic molecules, or can be administered to elicit their intrinsic therapeutic effects. Some of the first investigations of exosomes as therapeutic agents involved their use as non-cellular antigens for the development of vaccines against infectious diseases or tumor (Andre et al. 2002; Chaput et al. 2004; Wolfers et al. 2001). Exosomes carry both antigenic material and major histocompatibility complex peptide complexes, which are required in the antigen-presenting process of immune responses (Denzer et al. 2000). Presenting antigens, tumor-derived exosomes can evoke T cell activation in dendritic cells (DCs), resulting in glioma-specialized cytotoxicity (Chaput et al. 2004). More recently, it has been found that DCs exposed to tumor cell lysates release exosomes that elicit a T-cell immune response against intracranial glioma in mice, and significantly prolonged survival (Bu et al. 2015b).

In addition to their natural capacity to shuttle biomolecules and for immunomodulation, exosomes possess several characteristics that make them attractive therapeutic agents. Unlike transplanted cells, exosomes are non-viable, and thus, theoretically pose less safety risks, such as the formation of teratoma (Chen et al. 2011). Therapeutic exosomes may also be host-derived, circumventing any possible graft/host immune rejection (Chen et al. 2011; Sun et al. 2010). Furthermore, cells continually release exosomes in subculture, and the contents of exosomes remain stable for months ex vivo, thus large quantities can be manufactured at scale and stored (Chen et al. 2011). Given their nano-scale, exosomes pose little risk of embolism, and pass through the blood–brain barrier (BBB), making them useful for treatment of neurological diseases including brain tumor, where a characteristically leaky BBB may enhance delivery (Alvarez-Erviti et al. 2011; Anthony and Shiels 2013; Gheldof et al. 2013; Kooijmans et al. 2012; Meckes et al. 2013). Finally, the cargo and/or membranes of exosomes may be modified for tumor-specific, and possibly, patient-specific treatment. As a result, the potential for exosomes to be used as a vehicles to modify the immune response, or to selectively deliver therapeutic agents is fast drawing interest (Azmi et al. 2013; Johnsen et al. 2014; Momen-Heravi et al. 2014; Nazarenko et al. 2013; Sun and Liu 2014).

The current use of exosomes for therapy in treatment of primary brain tumor can be divided into three general categories: (1) exosomes for immunomodulation-based therapy, (2) exosomes as delivery vehicles for anti-tumor nucleotides, and (3) exosomes as drug delivery vehicles. An overview of current investigations using exosomes for treatment of brain neoplasms is provided in Table 1. In the three following sections, we will examine the current state of each of these nascent, yet fast-developing areas of research, and consider existing obstacles, open questions, and their future prospects.

Exosomes in Immunotherapy of Primary Brain Tumor

Cancer immunotherapy harnesses the body's own immune system to fight cancer. Unlike radiation, chemotherapy, and surgery, the immune system can potentially target tumor cells individually while sparing non-tumor cells, which makes it attractive for treatment of highly invasive brain tumors. Growing evidence indicates that a small cohort of highly tumorigenic, chemo- and radio-resistant stem-like tumor cells called cancer stem cells (CSCs) account for the high rates of tumor recurrence after treatment, as in glioblastoma, and immunotherapy carries the additional promise of being able to target CSCs specifically (Bao

Table 1 Experimental exosome-based treatments of brain tumor to date

Group	Exosomes employed	Loading method	Tumor	In vivo	Results
Munoz et al. (2013)	Mesenchymal stromal cell exosomes loaded with anti-miR-9	Cell/complex co-incubation then exosome harvest	U87, T98G glioblastoma	No	Exosomes conferred temozolomide chemosensitivity to tumor cells
Katakowski et al. (2012)	Mesenchymal stromal cell exosomes expressing miR-146b	MSC producer cells expressing miR-146b plasmid	9L gliosarcoma	Yes	Reduced intracranial tumor volume in Fisher rat
Yang et al. (2015)	Brain endothelial cell-derived exosomes loaded with doxorubicin	Exosome/drug co-incubation	U87 glioblastoma	Yes	Reduced tumor volume in exosome-delivered doxorubicin embryos compared to drug alone
Bu et al. (2015a, b)	Dendritic cell exosomes	Dendritic cells pulsed with chaperone-rich cell lysates then exosome harvest	GL261 glioma	Yes	Increased survival of mice, reduced tumor volume
Bronisz et al. (2014)	Exosomes from tumor cells expressing miR-1	Glioblastoma producer cells expressing miR-1 plasmid	U87, U251, U373, Gli36 glioblastoma	Yes	Reduced tumor growth, neovascularization, and invasiveness

et al. 2006; Cho et al. 2013; Salmaggi et al. 2006; Vik-Mo et al. 2013). Most immunotherapy of glioblastoma has employed dendritic cells, and has demonstrated some level of efficacy in phase I/II studies (Ardon et al. 2012; Okada et al. 2011; Sampson et al. 2009; Vik-Mo et al. 2013; Wheeler et al. 2008). However, strong phase III evidence for efficacy is lacking, and DC production is labor intensive and expensive (Bu et al. 2015a; Delcayre et al. 2005).

In 1996, Raposo et al. demonstrated that exosomes derived from B lymphocytes induced antigen-specific MHC class II-restricted T cell responses (Raposo et al. 1996). Two years later, Zitvogel et al., reported that tumor peptide-pulsed DC-derived exosomes (DEX) stimulated specific cytotoxic T lymphocytes in vivo and suppressed growth of murine tumors in a T-cell-dependent manner (Zitvogel et al. 1998). This, and more recent work, has revealed that DEX express MHC class I as well, and carry CD68, a potent co-stimulatory molecule that contributes to T cell priming during antigen presentation (Utsugi-Kobukai et al. 2003; Zitvogel et al. 1998). DEX have been show to transfer antigen-loaded MHC class I and II, and other associated molecules, to dendritic cells, resulting in amplification of the cellular immune response (Delcayre et al. 2005). Due to the immune-stimulating components and the advantages of non-cellular immunotherapy, DEX therapy has generated much interest, and therapeutic potential of DEX has been substantiated with their development and clinical testing in the treatment of metastatic melanoma, and non-small cell lung cancer (Delcayre et al. 2005; Escudier et al. 2005; Morse et al. 2005).

To date, investigations of DEX in treatment of brain tumor are relatively scarce. Only this year, Bu et al., reported that DCs loaded with DEX from glioma cell-derived chaperone-rich cell lysate-loaded DCs exhibit potent anti-tumor activity against intracranial glioma in mouse by promoting T-cell activation (Bu et al. 2015b). Recently, work by Romagnoli et al., revealed that breast adenocarcinoma cells incorporate DEX and subsequently express various surface molecules involved in the interaction between antigen-presenting cells and T lymphocytes (Romagnoli et al. 2015). Additionally, they demonstrated that these DEX-induced changes in tumor cells enhanced their ability to activate T cells to secrete IFN-γ (Romagnoli et al. 2015). Immunotherapy of glioblastoma has not produced clinical results comparable to those seen in prostate cancer or melanoma (Baxevanis et al. 2015; Delcayre et al. 2005; Singh and Overwijk 2015). This may be due in part that glioblastoma is considered particularly non-immunogenic. whereas prostate cancer which expresses well-characterized tumor-restricted antigens and melanoma is immunogenic (Delcayre et al. 2005; Pritchard et al. 2015; Singh and Overwijk 2015). Thus, while only speculative, the effect of increasing tumor cell immunogenicity might make DEX particularly advantageous for treatment of glioblastoma. Clearly, exosome-based immunotherapy for brain tumor is in its infancy. Even so, the brain is no longer considered impervious to tumor-specific immune cells, and exosomes will likely play a part in bringing immunotherapy to bear as a current standard of care for treatment of brain neoplasms.

Exosomes as Vehicles for the Delivery of Anti-tumor Nucleotides

Exosomes are enriched in ceramides, cholesterol, and sphingomyelin, which mediate cell-to-cell communication, and can transfer molecules between cells via membrane vesicle trafficking (Marsh and van Meer 2008; Penfornis et al. 2015, 2015). In 2007, Valadi et al. reported that human and mouse mast cell exosomes were carriers of over 1300 messenger mRNA and 121 non-coding miRNAs (Valadi et al. 2007). It is now known that most cells produce exosomes containing miRNAs, and that the majority of extracellular miRNA in human biologic fluids is contained within exosomes (Gallo et al. 2012; Qin and Xu 2014). By being encapsulated and contained within the exosome, bound RNA is protected from the digestion of RNAase (Valadi et al. 2007). miRNAs in exosomes can be transferred from cell-to-cell by exosome release and uptake, resulting in the intercellular regulation of mRNA translation by donated functional miRNAs which we reported in 2010 (Hu et al. 2012; Katakowski et al. 2010a; Mittelbrunn and Sanchez-Madrid 2012).

Exosomal miRNA does not necessarily reflect the miRNA profile within the parent cell (Nolte-'t Hoen et al. 2012; Ramachandran and Palanisamy 2012). Guduric-Fuchs et al. found that in HEK293T cells, expression of miR-146a enhanced export of miR146a, but relative expression of endogenous intracellular and extracellular vesicle miRNA was largely unaffected, suggesting that selective miRNA export is tightly regulated (Guduric-Fuchs et al. 2012). Indeed, Villarroya-Beltri et al. revealed that short sequence motifs over-represented in some miRNAs guide their binding to hnRNPA2B1, which controls their loading into exosomes (Villarroya-Beltri et al. 2013). Furthermore, the group demonstrated that directed mutagenesis could switch exosome-guided miRNA to be retained in the cell, or vice versa, suggesting that exosome miRNA cargo could be modified for therapeutic purposes by such mechanisms (Villarroya-Beltri et al. 2013).

Aberrant gene expression is the primary mechanism of miRNA dysfunction in most cancers, including glioblastoma, and miRNAs are differentially expressed in gliomas relative to normal tissue (Iorio and Croce 2012b; Nicoloso and Calin 2008; Silber et al. 2009; Zhang et al. 2012). Based on their efficiency in transferring functional miRNAs, interest in using exosomes as therapeutic miRNA delivery vehicles is growing. In 2011, Mittelbrunn et al. stably over-expressed miR-335 in J77 T-cells, which do not express miR-335, and demonstrated that the T-cell exosomes not only contained miR-335, but could transfer functional miR-355 to recipient cells which also did not express miR-355 (Mittelbrunn et al. 2012). These experiments demonstrated that cultured cells could be induced to package miRNAs into exosomes which might be used for a therapeutic effect. Shortly thereafter, we transfected primary marrow stromal cells (MSCs) with an expression plasmid for the miR-146b precursor, and found that they efficiently packaged mature miR-146b into MSC exosomes (Katakowski et al. 2012).

Glioblastomas display a variety of genotypes that are characteristically heterogeneous and unstable; thus the broad-signaling of miRNA is therapeutically advantageous when compared to the alteration of just one gene, and miRNA-based anti-tumor strategies have been suggested to be superior to other genotypic approaches (Fabbri et al. 2008; Iorio and Croce 2012a; Silber et al. 2009; Turner et al. 2010). Deletions on chromosome 10 are the most frequent chromosomal alteration observed in glioblastomas, and miR-146b is located within 10q24-26 (10q24.32), a region lost in most GBMs (Arslantas et al. 2007; Pershouse et al. 1993; Rasheed et al. 1992). We previously found that reconstitution of miR-146b expression significantly reduced malignancy of glioma cells with depleted miR-146b (Katakowski et al. 2010b). Therefore we posited that MSC exosomes carrying miR-146b might have an anti-tumor effect. To test this hypothesis, we over-expressed miR-146b in MSCs, harvested their exosomes (M146-exo), and intratumorally injected M146-exo into rats bearing intracranial 9L tumors (Katakowski et al. 2012). Here, we found that one intratumor injection of M146-exo 5 days after intracranial xenograft implantation significantly reduced tumor volume at 10 days post-implant compared to M67-exo or vehicle-treated control, or animals treated with exosomes containing cel-miR-67, a c. elegans miRNA with no known mammalian mRNA targets. These experiments indicate that exosomes can be tumor-suppressive when loaded with anti-tumor miRNAs. In an investigation with a similar approach, Shimbo et al., employed MSCs to package miR-143 into exosomes that could ectopically reduce migration of osteosarcoma cells; miR-143 is a tumor-suppressor miRNA that is characteristically down-regulated in the 143B human osteosarcoma cell line (Shimbo et al. 2014). Munoz et al., more recently reported that MSCs loaded with an anti-miR-9 nucleotide in non-contact co-culture with glioblastoma cells transferred the anti-miR to the glioma cells via secreted exosomes (Munoz et al. 2013). Interestingly, these exosomes conferred temozolomide chemosensitivity modulated by miR-9, suggesting that exosomes might play a role in the response to chemotherapeutics.

Although not employed as treatment per se, Bronisz et al., identified miR-1 deficiency as a contributor to growth, neovascularization, and invasiveness, and demonstrated that

reintroduction of miR-1 into glioblastoma microvesicles reverted paracrine-stimulated malignancy and microenvironmental remodeling by tumor (Bronisz et al. 2014). These findings support the hypothesis that miR replacement approaches have strong therapeutic potential, and can be mediated by extracellular vesicles. In addition, they raise the possibility that modified tumor exosomes might be employed as biological Trojan horses to suppress tumor cells and their effect upon the brain microenvironment.

In our experiments of primary brain tumor in rodent, miRNA-carrying MSC exosomes were injected directly into tumor, and distribution or miRNA delivery to non-tumor tissue was not determined. For clinical application, it would be ideal if therapeutic exosomes could be injected intravenously, or via intranasal administration, and if exosomes preferentially targeted tumor cells. To this end, Ohno et al. (2013) recently modified exosomes to target EGFR-positive breast cancer cells by expressing the transmembrane domain of platelet-derived growth factor receptor fused to the GE11 peptide, a ligand for EGFR (Ohno et al. 2013). Here the tumor-suppressor miRNA let-7 was over-expressed in HEK293 cells (an immortalized human embryonic kidney cell line), and packaged in GE11-positive exosomes (Ohno et al. 2013). Intravenous administration of let-7/GE11 exosomes reduced subcutaneous tumor growth, whereas untargeted let-7 exosomes did not (Ohno et al. 2013). Therefore systemic administration of anti-tumor exosomes can be improved by incorporation of tumor-specific peptides in the exosome membrane, a feature that might be important for efficient delivery from blood to tumor in brain. CSCs are considered to underpin tumor initiation and therapeutic resistance in glioblastoma (Heddleston et al. 2011). It is interesting to speculate whether exosomes loaded with tumor-suppressor miRNAs, targeted to glioma CSCs (i.e., by an antibody targeting the transmembrane CD133 glycoprotein), would elicit anti-tumor effects on par with the collapse CSC dynamics observed in previous investigations (Kang et al. 2014).

In addition to miRNA, exosomes can also be used to deliver interfering RNAs (siRNA) (Alvarez-Erviti et al. 2011; El-Andaloussi et al. 2012; Wahlgren et al.). However, unlike miRNAs, which are efficiently packaged into exosomes by producing cells, siRNAs must be incorporated by other means such as electroporation (Alvarez-Erviti et al. 2011; Wahlgren et al. 2012). Multiple groups have reported success with electroporation loading of nucleotides, however, in our own experience, electroporation loading efficiency was very low, which led us to conclude that technical hurdles to ensure efficient loading remain to be fully elucidated. A comprehensive study of exosome loading with siRNA by Kooijmans et al. (2013) suggests that electroporation of extracellular vesicles with siRNA is accompanied by extensive siRNA aggregate formation, which may result in overestimation of the amount of siRNA encapsulated (Kooijmans et al. 2013).

At the present moment, literature positing the use of exosomes as nucleotide delivery vehicles for treatment of tumor rivals reports of such investigations. Interest in exosomes as therapeutic agents is relatively new, and much about their nature and function has yet to be discovered. Exosomes contain molecular fingerprints of their parent cells, and initial investigations of exosomes in cancer were reasonably dominated by the promise of leveraging them for diagnostic purposes (Graner et al. 2009; Santiago-Dieppa et al. 2014; Skog et al. 2008). However, as an inherent function of exosomes is to deliver miRNAs over long distances in the body, it stands to reason that they can be harnessed to deliver therapeutic nucleotides. Current work indicates that exosome delivery of nucleotides may be optimized by enhanced packaging by producer cells, and by incorporation of tumor-targeting peptides to facilitate efficient delivery targeting exosomes to cancer cells. To our knowledge, our study with miR-146b-carrying MSC exosomes, and the recent work by Bronisz et al., are the only reported investigations of using exosomes to deliver anti-tumor miRNAs to brain tumor in vivo (Bronisz et al. 2014; Katakowski et al. 2012). Given the promise of miRNAs for improving therapy of glioblastoma, and the efficiency of exosomes to deliver them, further development of this treatment modality is warranted.

Exosomes as Drug Delivery Vehicles for Primary Brain Tumor

Like liposomes, exosomes possess a bilayer lipid membrane and an aqueous core, and therefore can also be loaded with both hydrophilic and lipophilic drugs. Exosomes have recently been tested as delivery vehicles for pharmaceutically active substances; however, to date there are even fewer reports than those for nucleotide delivery, and only one regarding treatment of brain tumor. Perhaps the first use of exosomes to deliver a drug was by Sun et al., who encapsulated curcumin in exosomes and demonstrated that the exosome-delivered curcumin protected mice against LPS-induced septic shock, by increasing solubility, stability, and bioavailability of the drug (Sun et al. 2010). Tian et al. (2014), have reported dendritic exosomes electroporation-loaded with doxorubicin were employed to treat breast xenograft tumors with significant anti-tumor effects (Tian et al. 2014). Of interest, tumor-targeting of exosomes was enhanced in their model by engineering the producer cells to express the membrane protein (Lamp2b) fused to αv integrin-specific iRGD peptide (Tian et al. 2014).

In brain, Yang et al. very recently reported the use of brain endothelial exosomes to deliver paclitaxel or doxorubicin across the BBB in a zebra fish model of brain tumor employing U87 glioblastoma (Yang et al. 2015). They found that when administered alone, doxorubicin and paclitaxel remained localized within the vasculature and did not penetrate the BBB, whereas exosome encapsulation facilitated delivery of the drugs across the BBB and reduced tumor progression (Yang et al. 2015). Therefore, exosomes might prove useful for the delivery of BBB-impermeable anti-cancer agents. Using the same methods as Sun et al. for encapsulated curcumin, Yang et al. loaded exosomes by simple co-incubation followed by isolation by centrifugation (Sun et al. 2010; Yang et al. 2015).

The ease by which chemotherapeutics can be delivered by exosomes is perhaps less surprising when one considers that tumor extracellular vesicles have been implicated in the clearance of chemotoxins from tumor cells (Safaei et al. 2005). As an example, cisplatin is removed by melanoma cells by secretion of intracellular organelles called melanosomes, which impair localization of drug to the nucleus (Chen et al. 2006). Challagundla et al. (2015), more recently revealed an exchange of miRNAs between neuroblastoma cells and peritumoral monocytes that underpins chemoresistance (Challagundla et al. 2015). In 2003 Shedden et al. performed profiling experiments on the NCI panel of 60 cancer cell lines that revealed inverse correlation between chemosensitivity and expression of genes related to vesicle secretion suggesting that exosomes play a natural role in export of cytotoxic substances (Shedden et al. 2003). Further studies are warranted to determine if exosome-encapsulated chemotherapeutics are more or less effective at treating chemoresistant cells, and if exosome-delivered drug can be similarly expulsed. The alkylating agent temozolomide (TMZ) is the most commonly used compound for treatment of glioblastoma. There are yet no studies that employ exosomes for delivery of TMZ, and such experiments merit investigation.

Considerations for Further Development of Exosome Treatment of Primary Brain Tumor

Interest is growing in using exosomes for treatment of tumor for a number of reasons: (1) exosomes released from donor cells are taken up by acceptor cells, where biological processes, including cell proliferation and differentiation, apoptosis, and immune response, can be affected (Hu et al. 2012), (2) compared to cellular RNA, exosomal RNA is more stable (Keller et al. 2011), (3) exosomes are resistant to degradation during prolonged storage and in freeze/thaw cycles (Chen et al. 2011), (4) administered exosomes do not elicit acute immune rejection (Chen et al. 2011), (5), exosomes are non-viable, and do not risk tumor formation, or reprogramming by tumor (Chen et al. 2011; Hu et al. 2012), (6) unlike viral vectors, exosome delivery does not result in undesired extended expression of genes, (7) in culture, exosomes can be manufactured at scale, and possibly using autologous cells (Chen et al. 2011), (8) exosome-cargo and/or membranes can be tailored for tumor-specific, or personalized treatment, and (9) exosomes can pass the blood–brain barrier, which makes them attractive delivery vehicles for treatment of neurological disease, including brain malignancies (Xin et al. 2014).

Accumulating evidence suggests a pattern of deregulated miRNAs in human glioblastoma (Barbano et al. 2014; Piwecka et al. 2015). Numerous reports indicate the potential of miRNA for treatment of brain tumor as miRNA-based therapies produce uniquely potent anti-tumor effects due to their actions on multiple oncogenic pathways (Gabriely et al. 2011; Iorio and Croce 2012a; Papagiannakopoulos et al. 2008). Exosomes are now recognized as efficient carriers for functional miRNAs. Thus, exosomes have considerable promise as new delivery vectors for tumor-suppressive miRNAs to treat malignant glioma (Hagiwara et al. 2014).

Key to development of exosome therapeutics is a better understanding of the clearance, tumor-targeting, and biodistribution of administered exosomes. Smyth et al. (2015) recently noted rapid clearance and minimal tumor accumulation of intravenously administered unmodified exosomes; however, when delivered intratumorally, exosomes remained associated with tumor to a greater extent than phosphatidylcholine-cholesterol liposomes (Smyth et al. 2015). Exosomes can pass the BBB; yet for treatment of invasive brain tumor, targeted delivery is critical, lest individual cells that escape therapy lead to tumor recurrence. Substantial investigation of the delivery of exosomes to tumor in brain is needed. In our experimental treatment of intracranial glioma, MSC exosomes were injected intratumorally (Katakowski et al. 2012). Viral vectors, proteins, and cells have been administered by intratumoral injection or injection into the brain adjacent to the tumor cavity during surgical resection, and exosomes might be similarly administered (Dillman et al. 2004; Geletneky et al. 2012; Markert et al. 2009; Oshiro et al. 2006). Convection-based delivery is being used clinically to treat recurrent glioblastoma, and shows promise in delivering drug-loaded polymeric nanoparticles (Barua et al. 2014; Zhou et al. 2013). Exosomes may be well-suited for convection-based delivery, and may be cleared from brain less efficiently than low-affinity liposome formulations (Barua et al. 2014; Johnsen et al. 2014; Saito et al. 2006). Work by Ohno et al. (2013) and Rana et al. (2012) suggests that delivery of exosomes can be enhanced with tumor-targeting membrane peptides; thus, intravenous or intranasal

routes of delivery might be viable for brain tumor as well (Ohno et al. 2013; Rana et al. 2012).

Another important decision in the development of exosome therapy is the choice of manufacturing cells. Of the cell types known to produce exosomes, MSCs are seen as the most prolific producer (Yeo et al. 2013). MSCs can be extracted and isolated relatively easily (harvested in a bone marrow aspiration via the posterior iliac crest), and in some cases, it may be advantageous to use the patient's own cells to produce exosomes for therapy. Even so, MSC exosomes appear to be non-immunogenic, if not immunosuppressive, and graft/host risks are likely minimal (Tan et al. 2014; Yeo et al. 2013). Chen et al., demonstrated that MSC cells immortalized by MYC-transformation produced more exosomes with similar therapeutic efficacy as those produced by untransformed MSCs, indicating that immortalized producer lines might be a low-cost and efficient alternative to primary MSC harvest (Chen et al. 2011). However, tumor-derived exosomes can possess immunosuppressive and tumor-supportive properties (Kahlert and Kalluri 2011; Yang and Robbins 2011). In addition, Balaj et al. (2011), found that tumor microvesicles contain retrotransposon elements, and can mediate horizontal gene transfer (Balaj et al. 2011). Such characteristics may limit the utility of tumor cells or immortalized cells for exosome production. Exosomes exhibit properties and functions of their parent cells. Therefore, it is clear that production capacity should not be the only selection characteristic for producer cells. In our MSC exosome treatment of intracranial glioma, naive MSC exosomes did not increase growth compared to PBS control (Katakowski et al. 2012). However, MSC exosomes exhibit intrinsic neurogenerative properties, which may or may not frustrate their application for the treatment of brain tumor (Xin et al. 2012; Zhang et al. 2015). As an example, Ono et al., have shown that exosomal miRNAs from the bone marrow may promote breast cancer cell dormancy (Ono et al. 2014). While MSC exosomes may be ideal vehicles for neurorestorative treatments, it may be that DEX represents a better exosomal vehicle for anti-tumor nucleotides or chemotherapeutics for the treatment of brain malignancies. DEX, can harbor anti-tumor T cell activation properties, as well as the ability to induce immunogenicity of tumor cells, as discussed (Romagnoli et al. 2015; Zitvogel et al. 1998).

Exosomes mediate long-range transfer of signaling molecules without inducing adverse immune reactions and they can pass the BBB, which makes them attractive candidates for delivery to brain tumor (41–43). Exosomes may be engineered to carry a variety of molecules including therapeutic nucleotides, pharmaceuticals, and/or protein or peptides (Katakowski et al. 2012; Lai et al. 2013; Lee et al. 2012; Mathivanan et al. 2010). Finally, dendritic cell-derived exosomes may be used to stimulate immunorejection of tumor (Bu et al. 2015b). Successful development of exosome-based anti-tumor therapy demands deliberate and tested procedures for selection of exosomes, conditions under which they are produced, modification of the exosomes or their parent cells, and route of administration. As two examples, the common isolation protocol using differential centrifugation and a sucrose gradient yield a heterogeneous product, and fetal bovine serum used in many media formulations contain exosomes that substantially influence cultured cell behavior (Lai et al. 2013; Lee et al. 2012; Shelke et al. 2014). Despite early promising experimental results, the use of exosomes for treatment of primary brain tumor is far from a clinical reality. Nevertheless, given the propensity of exosomes to deliver small molecules, and recent recognition of their importance in tumorigenesis and progression, further work on developing exosome-based therapeutics for brain tumor is certain to be worthwhile.

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