Review Article

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Mesenchymal stem cell exosomes: a promising delivery system for glioma therapy

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Abstract: Gliomas are particularly challenging due to their high invasiveness, frequent recurrence, and elevated mortality rates. Despite the availability of treatments like surgery, radiation, and chemotherapy, each of these methods faces significant limitations. This has led to a pressing demand for new strategies against gliomas. In this landscape, mesenchymal stem cells (MSCs) have shown significant potential in recent years. However, the application of MSCs in glioma therapy encounters various challenges. A significant advancement in this field is the utilization of exosomes (Exo), key secretions of MSCs. These exosomes not only carry the benefits inherent in MSCs but also exhibit unique physicochemical properties that make them effective drug carriers. Consequently, MSCs Exo is gaining recognition as a sophisticated drug delivery system, specifically designed for glioma treatment. The scope of MSCs Exo goes beyond being just an innovative drug delivery mechanism; it also shows potential as a standalone therapeutic option. This article aims to provide a detailed summary of the essential role of MSCs Exo in glioma progression and its growing importance as a drug delivery carrier in the fight against this formidable disease.

Keywords: mesenchymal stem cells; exosomes; engineered exosomes; exosome-based drug delivery; glioma treatment

Introduction

Gliomas, as the predominant primary brain tumors within the central nervous system, account for 80 % of all primary brain malignanciesm [1]. Among these, the World Health Organization (WHO) grade IV glioblastoma multiforme (GBM) stands out as the most aggressive form, characterized by a median survival time of merely 15 months [2]. Despite advancements in surgical techniques, radiation therapy, and chemotherapy, the prognosis for glioma patients remains grim [3]. These therapies face several limitations: the impossibility of completely excising the tumor surgically, the risk of radiation-induced damage to adjacent healthy brain tissue, and significant challenges such as the difficulty for chemotherapy drugs to penetrate the blood–brain barrier (BBB) and the emergence of resistance to chemotherapy [4, 5]. In light of these constraints, the focus of research is shifting towards MSCs as an innovative therapeutic strategy to overcome the shortcomings in glioma treatment [4, 6]. A key attribute of MSCs is their natural tendency to home to tumor sites, a characteristic that could be exploited for targeted therapy [7]. Once localized at the tumor, MSCs exhibit anti-tumor activities by suppressing tumor cell growth and promoting apoptosis [6, 8, 9]. However, the interaction with the tumor microenvironment (TME) can influence MSCs to develop a pro-tumor phenotype, potentially exacerbating tumor progression [10, 11]. This dual nature of MSCs in the tumor context makes their role highly controversial. Furthermore, MSC-based treatments are not just about cell-to-cell contact but also involve the release of extracellular vesicles (EVs), notably exosomes. These exosomes play a crucial role in modulating the immune response and reshaping the microenvironment, fostering conditions favorable for tissue repair and potentially counteracting tumor growth [8, 12].

Exo represents small vesicles excreted by a multitude of cell types, containing proteins, RNA, DNA fragments, lipids, and metabolites, they can migrate to target cells through diverse mechanisms, modulating their functional responsiveness [13–15]. In recent years, exosomes have emerged as promising candidates for glioma treatment. These vesicles are particularly effective in directly delivering chemotherapeutic drugs, such as Doxorubicin (DOX) and Paclitaxel (PTX), to tumor sites [16–18]. This targeted delivery not only reduces chemoresistance but also minimizes the systemic side effects typically associated with

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such treatments. Further enhancing their therapeutic potential, engineered exosomes can be modified to express miRNA, mRNA, and siRNA, which are instrumental in inhibiting glioma cell proliferation, invasion, and migration [19–23]. Gliomas, characterized by high vascularization, can also be targeted through the anti-angiogenic properties of exosomes [24, 25]. Overcoming drug resistance, a significant hurdle in effective glioma treatment is another area where engineered exosomes show promise. By enhancing the sensitivity of tumor cells to chemotherapeutic agents, these exosomes can play a crucial role in treatment efficacy [26–28]. Additionally, the immunosuppressive microenvironment of gliomas contributes to their heterogeneity and treatment resistance. Here, exosomes can exert an immunomodulatory effect, reshaping this microenvironment and counteracting tumor invasion [29–31]. Despite their potential, one of the challenges with exosomes is their relatively low targeting ability. Recent research has focused on engineering modifications to improve their specificity towards glioma cells [26, 32–37]. Moreover, combining exosome-based therapies with other physical treatment methods, such as focused ultrasound and local magnetic positioning, shows promise in increasing their BBB penetration and accumulation within the brain, thereby enhancing therapeutic outcomes [34, 38, 39].

MSCs Exo display both tumor-promoting and suppressing functions [40, 41]. The outcomes of MSCs Exo's actions might depend on their origin, constituents, and the tumor's stage. Their potential as a drug delivery system is significant, especially when compared to conventional nanocarriers, as they can cross the BBB, are stable, and have reduced immunogenicity and toxicity [42–44]. However, the challenge of targeting precision remains. In summary, this article reviews the role of MSCs Exo in glioma progression and their potential as a drug delivery system. It highlights the advancements in using MSCs Exo for glioma treatment, offering insights into their application in this challenging field.

Mesenchymal stem cells and exosomes

Mesenchymal stem cells

MSCs are multipotent, non-hematopoietic progenitor cells found in tissues like adipose, bone marrow, dental pulp, umbilical cord, and placenta [45]. These cells can differentiate into various cell types, including osteocytes, chondrocytes, and adipocytes [46]. Notably, MSCs can modulate tumor cell proliferation and immune responses [47], exerting either suppressive or promotive effects on tumor progression [10, 48]. They target multiple components in the TME, including immune cells, endothelial cells, and fibroblasts [49], as depicted in Figure 1. Additionally, MSCs can transform into tumor-associated MSCs (TA-MSCs), adopting a tumor-supportive phenotype that facilitates tumor growth [10, 11]. Despite their dual role in influencing tumor cells, the mechanisms underlying MSCs' functional transformation and their homing to tumors remain areas of active research. Some studies suggest that MSCs are drawn to tumors by interactions between specific cytokines and chemokine receptors, such as SDF-1/CXCR 4, SCF-c-Kit, HGF/c-Met, VEGF/VEGFR, PDGF/PDGFR, and MCP-1/CCR 2 [50]. Their low immunogenicity and strong tumor tropism, coupled with ease of acquisition and rapid proliferation, make MSCs promising carriers for anti-tumor biotherapeutics, including cytokines, chemotherapeutic agents, and oncolytic viruses [51, 52].

However, tissue-derived MSCs face challenges such as donor variability and limited scalability. An alternative source of MSCs is embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). However, ESCs pose significant immune rejection risks and ethical concerns, while iPSCs are plagued by genetic instability. Human embryonic stem cells (hESCs) can differentiate into MSCs, producing uniform mesenchymal tissues without teratoma formation and can further differentiate *in vitro* [53–55]. These hESC-derived MSCs (hES-MSCs) offer advantages in scalability and quality consistency and may have enhanced immunomodulatory functions compared to MSCs [56]. In tumor-bearing mice, the direct injection of hES-MSCs engineered with adenovirus and lentivirus vectors into xenografts or into the contralateral hemisphere can inhibit tumor growth and prolong survival time [57]. The differentiation of iPSCs into MSCs is currently a highly researched area. Utilizing a non-viral, non-integrating reprogramming platform to generate iPSCs results in more stable pluripotent cells, which can then differentiate more effectively. This method leads to the production of iPSC-derived MSCs (iMSCs) that not only tackle the challenges of heterogeneity and scalability associated with traditional MSCs but also retain their significant therapeutic benefits [58, 59]. iMSCs have demonstrated effectiveness in inhibiting tumor proliferation and metastasis in various cancer models [60–62].

MSCs are derived from a variety of tissues, including adipose tissue, bone marrow, dental pulp, umbilical cord, and placenta. These cells exhibit the potential to differentiate into diverse cell types such as osteocytes, chondrocytes, and adipocytes. In the TME, MSCs interact with different cellular components including immune cells, endothelial

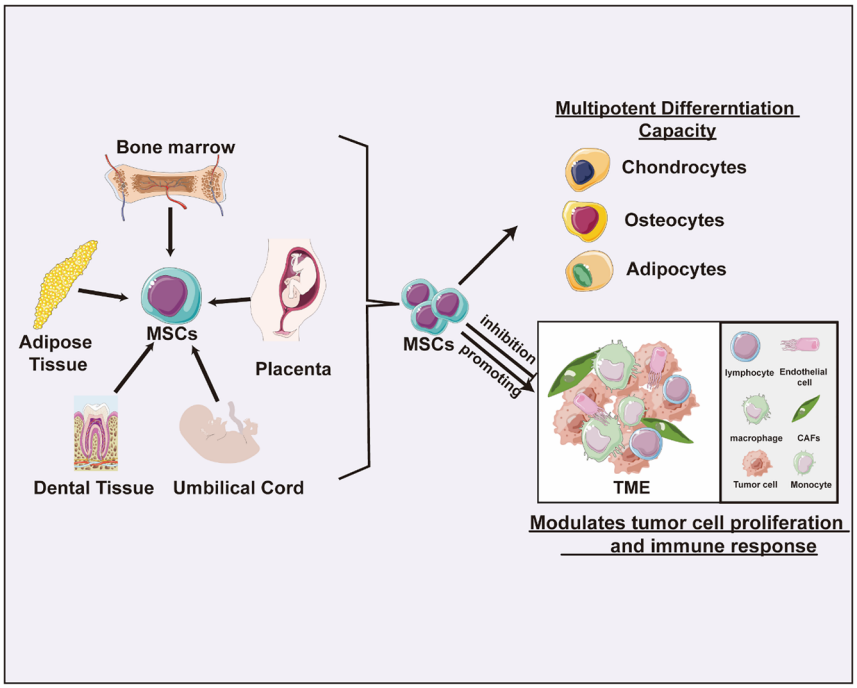

Figure 1: Origin, differentiation, and effects of MSCs on the TME (the figure was drawn using AI and partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license).

cells, and tumor-associated fibroblasts. These interactions are crucial in modulating the proliferation and immune responses of tumor cells. MSCs play a dual role in tumor progression, where they can either suppress or promote tumor growth, depending on various factors in the TME.

Exosomes and their advantages as drug delivery vehicles

Exosomes, a major type of EVs, are secreted by all cell types and contribute to intercellular communication. These vesicles vary in size, biogenesis, and content, encapsulating a range of biomolecules such as signaling molecules, RNA, proteins, DNA fragments, carbohydrates, and lipids [63]. EVs are ubiquitous in bodily fluids including urine, blood, tears, saliva, cerebrospinal fluid (CSF), and breast milk [64], and play a critical role in regulating cell function, morphology, and outcomes through various signaling pathways upon reaching their target cells. According to the International Society for Extracellular Vesicles (ISEV), EVs are classified into three subtypes: exosomes (30–150 nm), microvesicles (100–1,000 nm), and apoptotic bodies (1,000–6,000 nm), distinguished by their size and biogenesis [63]. Originating within multivesicular bodies (MVBs), exosomes are released into the extracellular matrix and can target both nearby and distant cells, significantly influencing cellular processes in both normal and pathological conditions [65–67].

Exogenous nanomaterials, commonly used as drug carriers, face challenges such as triggering autoimmune responses, causing thrombosis, generating cytotoxic effects, and low clearance rates in organs [68]. These issues limit their clinical application. In contrast, Exo offer advantages due to their endogenous nature, providing greater stability than synthetic polymers and liposomes. Exo exhibits properties conducive to immune surveillance, possesses an extended half-life, and shows potential in targeting receptor cells. Its ability to cross the BBB and carry diverse molecules like proteins, lipids, RNA, and DNA fragments is notable [69–71]. As such, Exo is emerging as a superior vehicle for the systemic delivery of targeted drugs. Encapsulation of antitumor drugs in exosomes enhances drug solubility, bioavailability, and stability, while reducing rapid drug clearance and unintended tissue deposition [72]. However, the efficiency of exosome targeting in GBM is limited, as intravenously administered Exo predominantly accumulates in the spleen and liver, with only a small fraction reaching the tumor site [73]. To overcome this, engineered Exo with tumor-targeting capabilities can be developed, enhancing their accumulation at tumor sites and marking a novel trend in translational medicine [74].

Mesenchymal stem cell-derived exosomes

MSCs, recognized as a pivotal source for clinical cell therapy, are widely considered the ideal carriers for anti-cancer

biological agents. Despite their potential, utilizing MSCs as therapeutic carriers presents challenges, including unstable differentiation, potential vascular clotting, and infection risks. Exosomes, key secretions of MSCs, inherit many of their parent cells' advantages [75]. These exosomes, thanks to their unique physicochemical properties, can surmount the limitations of MSCs in drug delivery applications. It is noteworthy that MSCs produce a higher quantity of exosomes compared to other cell types [76], and these exosomes demonstrate strong tumor-targeting capabilities [77, 78], reduced immunogenicity [79], and an enhanced ability to penetrate the BBB [80].

Encapsulation of chemotherapy drugs using MSCs Exo significantly enhances their ability to inhibit tumor proliferation and progression, while maintaining high biosafety. For instance, drugs delivered via MSCs exosomes are less likely to cause myocardial damage compared to free chemotherapeutic drugs [81]. MSCs have inherent tumor-tropic properties, which are believed to be due to their ability to home to tumor sites in response to inflammatory signals [82]. This characteristic is exploited to use MSC-derived exosomes as vehicles for drug delivery to tumor sites, including gliomas. The specificity of these exosomes in targeting tumor cells arises from multiple aspects: (1) surface proteins: exosomes express specific surface proteins that interact with receptors or molecules uniquely or overexpressed on tumor cells [83]; (2) microenvironmental factors: the tumor microenvironment, including hypoxia and inflammatory signals, may modulate exosome uptake by tumor cells [78, 84]; (3) genetic and molecular targeting: exosomes can be engineered to carry molecules like small siRNA, miRNA, circRNA, lncRNA, or specific drugs that target molecular pathways critical for tumor cell survival [85–89]. Additionally, further enhancing MSC-Exo's tumor-targeting by jointly altering surface properties and contents through covalent modifications or genetic engineering shows their potential from basic research to clinical application [83, 90, 91].

The effects and underlying mechanisms of MSC-Exo on glioma progression

Tumor-promoting effects

Research has established that exosomes from MSCs can accelerate glioma progression (refer to Figure 2). **Figueroa et al**. identified a novel element in the glioma matrix, termed Glioma Associated-human Mesenchymal Stem Cells (GA-HMSCs). These cells, isolated from surgical specimens and used within five generations in experiments, secrete exosomes that carry miR-1587 to Glioma Stem Cells (GSCs), leading to a reduction in nuclear receptor corepressor 1 (NCOR1) expression. This interaction notably increases GSC proliferation, clonality, and tumorigenicity in both *in vitro* and *in vivo* environments, underscoring the vital role of GA-HMSCs in tumor support and the significance of tumor-stroma interactions in tumor development [92]. Extending this research, **Qiu et al.** generated Glioma Associated Mesenchymal Stem Cells (GA-MSCs) from bone marrow-derived MSCs (BM-MSCs) from both mice and humans. They discovered that exosomal miR-21 from GA-MSCs boosts CD73 expression in myeloid-derived suppressor cells (MDSCs). CD73, functioning as an ectonucleotidase, fosters an immunosuppressive environment via adenosine production. Moreover, subsequent investigations showed glioma-derived exosomal CD44 triggers the miR-21/SP1/DNMT1 feedback loop in MSCs. This increases miR-21 levels in MSCs exosomes, thereby amplifying the immunosuppressive effects of glioma exosomes. Interestingly, the study suggests that modified dendritic cell-derived exosomes carrying miR-21 inhibitors could target GA-MSCs and reduce CD73 expression on MDSCs, potentially synergizing with anti-PD-1 monoclonal antibody therapy. This indicates that while GA-MSCs can promote glioma growth and immunosuppression, there is potential for therapeutic intervention through the modulation of exosomal content [93].

Specifically, MSCs Exo miR-1587 targets and reduces the levels of NCOR1. This reduction is directly associated with a significant increase in both the growth and clonality of GSCs. Additionally, MSCs play a crucial role as signal amplifiers. They intensify the immunosuppressive effects exerted by glioma exosomes, thereby contributing substantially to the progression of glioma.

Tumor-suppressing effects

Recent research, as depicted in Figure 3, suggests that MSCs-Exo might play a role in hindering glioma progression. Parsaei et al. conducted a study where C6 cells were co-cultured with exosomes from varying concentrations of rat bone marrow mesenchymal stem cells (rBMMSCs). Their findings revealed that these exosomes predominantly induce cell death by promoting apoptosis, and at the same time, they noted a direct linear correlation between exosome concentration and cytotoxicity [94]. Further supporting these findings, **Xu** and colleagues discovered that exosomes from mouse BM-MSCs, containing miR-133b, significantly inhibit the expression of EZH2. This inhibition

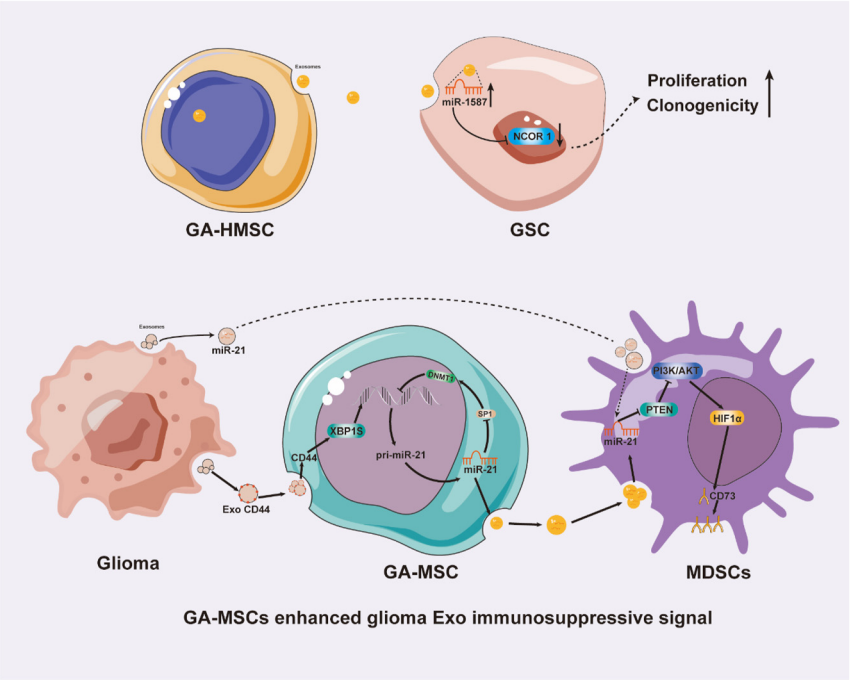

Figure 2: The promotive effects of exosomes from MSCs on glioma (the figure was drawn using AI and partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license).

likely suppresses the Wnt/β-catenin signaling pathway, thereby reducing proliferation, migration, and invasion of glioma U87 cells *in vitro*. Complementary *in vivo* studies also demonstrate the capability of these exosomes to impede the progression of gliomas [95]. Additionally, research has indicated that long non-coding RNA PTENP1, encapsulated in exosomes from human umbilical cord mesenchymal stem cells (hUC-MSCs), plays a crucial role. It binds competitively with miR-10a-5p, stabilizing PTEN, which leads to the inhibition of U87 cell proliferation and promotion of apoptosis [96]. **Yu et al.** reported that exosomes from human MSCs (hMSCs) can transport miR-199a to U251 glioma cells. This transport inhibits cell proliferation, invasion, and migration by modulating AGAP2 expression. Moreover, hMSCs overexpressing miR-199a significantly enhanced the chemosensitivity to temozolomide and curtailed *in vivo* tumor growth [97]. Table 1 presents a summary, encompassing the origins and passage

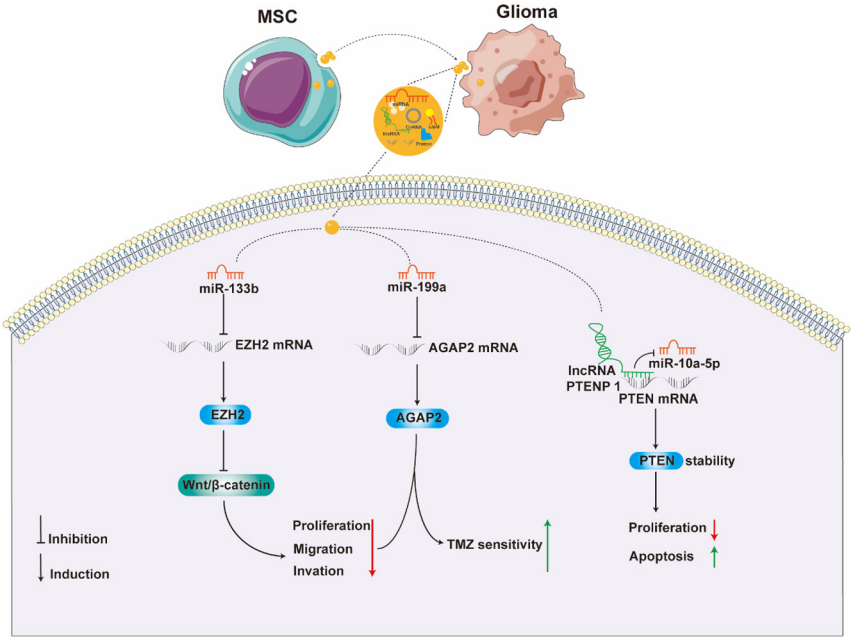

Figure 3: Inhibitory effects of MSCs Exo on glioma (the figure was drawn using AI and partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license).

Table 1: Effects and related mechanisms of MSCs Exo on glioma ("N/A" indicates not mentioned or not applicable).

Donor cell	Receptor cell	Passage number of cell	Exo cargo	Expression	Mechanisms/ targets	Function	Study model	Ref.
GA-HMSCs	GSCs	Within five generations	miR-1587	Up	NCOR1	Increase tumorigenicity of GSCs	*In vitro* and *in vivo* (GSCs-nude mice *in situ* tumor formation)	[92]
GA-MSCs	MDSCs	N/A	miR-21	Up	CD73	Amplifying immunosuppressive signals	*In vitro* and *in vivo* (GL261-C57BL/6 *in situ* tumor formation)	[93]
rBMMSCs	C6	The third passage	N/A	N/A	N/A	Promote c6 cell apoptosis, inhibit migration and invasion	*In vitro*	[94]
MSCs	U87	The third passage	miR-133 b	N/A	Wnt/β-catenin-EZH 2	Inhibit glioma cell proliferation, migration and invasion	*In vitro* and *in vivo* (U87--nude mice subcutaneous tumor formation)	[95]
hUC-MSCs	U87	Within 8 passages	lncRNA PTENP 1	N/A	miR-10a-5 p/PTEN	Promote U87 cell apoptosis, inhibit proliferation	*In vitro*	[96]
hMSCs	U251	The third passage	miR-199 a	N/A	AGAP 2	Inhibit glioma cell proliferation, migration and invasion; enhance the chemosensitivity of temozolomide	*In vitro* and *in vivo* (U251-Balb/c nude mice subcutaneous tumor formation)	[97]

numbers of MSCs, as well as an overview of the roles, underlying mechanisms, and experimental models associated with MSCs-Exo in the context of glioma research.

MSC Exo is capable of transporting miRNA or lncRNA, which through modulation of the expression or stability of EZH2, AGAP2, and PTEN, impacts the apoptosis, growth, migration, invasion, and drug tolerance of glioma cells, constraining glioma advancement.

Advancements of MSCs Exo in glioma therapy

Introduction to exosome drug loading methods

As depicted in Figure 4, there are two primary methods for loading therapeutic drugs into MSCs Exo: (1) the direct method involves encapsulating drugs into exosomes using various techniques; (2) the indirect method includes generating exosomes that carry different biomolecules (like nucleic acids, proteins) through genetic engineering or by co-incubating cells with therapeutic drugs. For direct drug encapsulation into exosomes, techniques such as electroporation, incubation, extrusion, ultrasonication, saponification, and freeze-thaw cycles are employed. Incubation, being the most common method, is simple but has relatively low efficiency in drug loading. Electroporation, in contrast, is more efficient but risks disrupting exosome structure due to electric field-induced protein or RNA clusters, potentially reducing drug delivery effectiveness [98]. Ultrasonication is noted for its high efficiency, but it can also damage exosome structure and lead to protein aggregation [99]. While ultrasonication is more damaging to exosomal integrity compared to other methods [100]. Extrusion stands out by producing uniform exosomes, thereby enhancing drug delivery efficiency [101]. However, improper mechanical pressure during extrusion can harm the exosome structure [102]. Freeze-thaw cycles, commonly used in drug delivery systems, may alter the physicochemical properties of the exosome membrane and are less efficient than ultrasonication in drug loading [101, 102]. Currently, incubation and electroporation are the most frequently used techniques for drug loading in MSCs Exo. With their notable drug delivery advantages, MSCs Exo have been extensively utilized in tumor treatment strategies.

Two primary techniques are employed to produce exosomes containing therapeutic agents: the direct and indirect methods.

A. The indirect method involves either incubating cells with drug molecules or using transfection or transduction with expression vectors. These approaches prompt cells to release Exo that carry drug molecules, viral proteins, nucleic acids, and proteins.
B. The direct method starts with isolating exosomes. Subsequently, therapeutic drugs are loaded into these Exo either through passive incubation or active methods

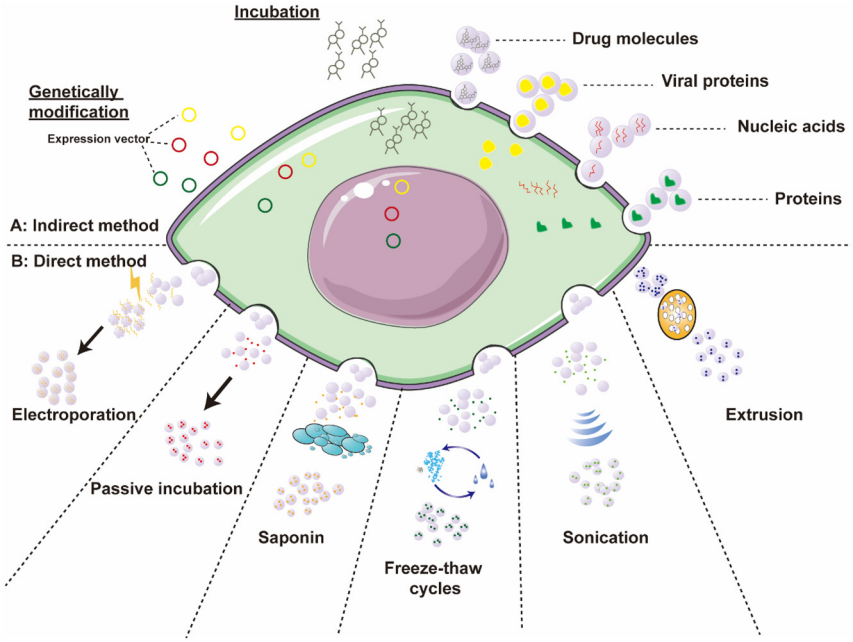

Figure 4: Schematic diagram of exosome drug loading techniques (the figure was drawn using AI and partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license).

such as electroporation, saponin treatment, freeze-thaw cycles, ultrasonication, and extrusion.

Treatment of glioma with drug-loaded MSCs exo

Del Fattore et al. successfully loaded vincristine into MSC EVs derived from the umbilical cord using a co-incubation method with drugs and cells. Their research demonstrated a notably increased cytotoxic effect on U87 glioblastoma cells compared to both free vincristine and unloaded EVs, thereby confirming the efficacy of MSC EVs in delivering anti-tumor drugs directly to glioblastoma cells [103]. Additionally, researchers investigated the loading of various concentrations of atorvastatin into exosomes from human endometrial mesenchymal stem cells (hEnMSCs) using an incubation technique. These exosomes were then co-cultured with 3D spheroids of U87 glioblastoma and human umbilical vein endothelial cells (HUVECs). The findings indicated that hEnMSCs-Exo loaded with atorvastatin significantly inhibited angiogenesis and tumor migration and proliferation. Notably, higher concentrations of atorvastatin in the exosomes resulted in more pronounced anti-tumor effects [25] (Figure 5A). In conclusion, encapsulating anti-cancer drugs in exosomes not only enhances their solubility, bioavailability, and stability but also prevents rapid drug degradation and undesirable distribution to various tissues. Due to their nano-sized dimensions, exosomes are capable of crossing the blood-brain barrier, enabling efficient and targeted drug delivery to the brain. Consequently, exosomes loaded with anti-tumor drugs, such as those from MSCs, represent a highly promising and effective approach for glioblastoma therapy, warranting further investigation.

MSCs Exo-mediated overexpression of miRNA in glioma therapy

Enhance the sensitivity of chemotherapy or anti-cancer agents

Sharif et al. discovered that miR-124, transported via exosomal mechanisms or independently, can be successfully delivered to U87 GBM cells in conjunction with Wharton's jelly mesenchymal stem cells (WJ-MSCs) from the human umbilical cord. This process targets cyclin-dependent kinase 6 (CDK6), thereby enhancing the chemosensitivity of U87 cells to temozolomide (TMZ) and reducing their migration [27]. In the context of TMZ resistance, miR-9 is upregulated in GBM cells and contributes to the expression of the drug efflux transporter P-glycoprotein (P-gp). To mitigate miR-9's role in promoting drug resistance, a Cyanine 5(Cy5)-labeled anti-miR-9 strategy was employed. This research revealed that exosomes from hMSCs were crucial in the delivery of anti-miR-9. Additionally, these hMSC-derived exosomes, when overexpressing anti-miR-9, effectively reduced P-gp levels, reversing TMZ resistance in U87 and T98G GBM cells [28]. TNF-related apoptosis-inducing ligand (TRAIL) has emerged as a promising anticancer agent. It's been shown

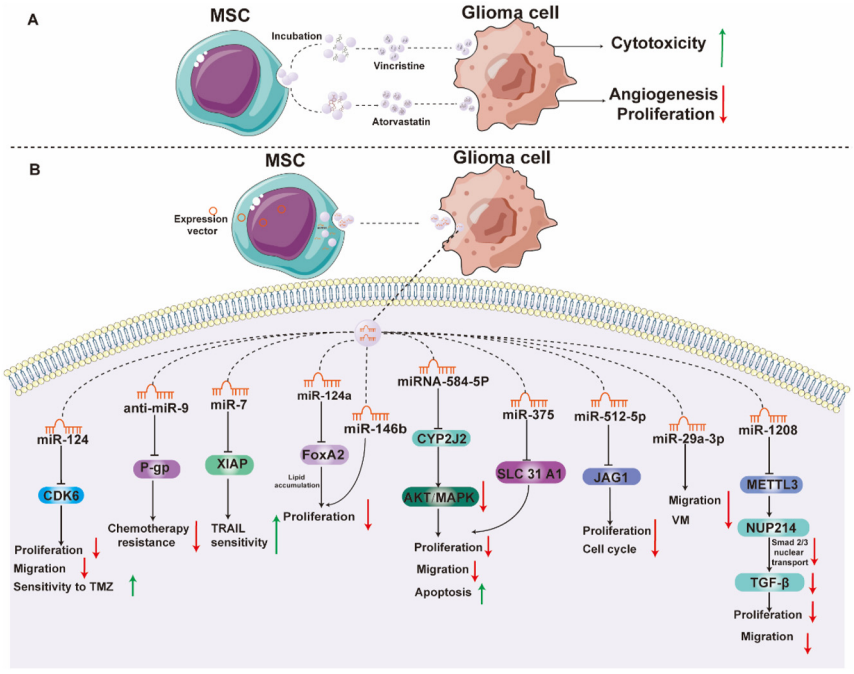

Figure 5: Drug loading and miRNA overexpression in MSCs exosomes for glioma treatment (the figure was drawn using AI and partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license). (A) Drugs are loaded into MSCs exosomes through co-incubation, influencing gliomas' proliferation, angiogenesis, and cytotoxic response. (B) miRNA overexpression in exosomes, facilitated by plasmid transfection, impacts glioma characteristics such as drug resistance, proliferative capacity, migration ability, apoptosis efficiency, and angiogenic potential.

that therapeutic miR-7, overexpressed in TRAIL-BMMSCs and loaded into cell-derived exosomes, enhances TRAIL sensitivity by targeting and suppressing X-linked inhibitor of apoptosis protein (XIAP), leading to significant apoptosis in U87 cells. Moreover, *in vivo* studies highlight that mouse BMMSCs co-expressing TRAIL and miR-7, through their exosomes, synergize to exert an anti-tumor effect [104].

Inhibition of tumor proliferation and migration

Lang et al. utilized a miR-124a lentiviral vector to transduce human MSCs derived from bone marrow. They co-cultured these cells with GSCs using isolated MSCs-Exo-miR124, which significantly reduced the viability and clonogenicity of GSCs. When used to intervene in GSC267-bearing nude mice, MSC s-Exo-miR124 enabled 50 % of the mice to achieve long-term survival. Mechanistic studies revealed that miR-124a suppresses tumor proliferation by silencing Forkhead box A2 (FoxA2), leading to abnormal intracellular lipid accumulation [21]. Katakowski's work involved transfecting rat bone marrow-derived MSCs with miR-146b expression plasmids. By co-culturing overexpressing miR-146b MSCs derived exosomes with 9L cells, the growth ability of 9L cells was reduced. Further, intratumoral injection of these exosomes in *in situ* brain tumor rat models significantly diminished the growth of glioma xenografts [22]. Kim et al. discovered that hMSCs Exo overexpressing miRNA-584-5P inhibit u87 cell proliferation and migration while promoting apoptosis. This effect is achieved by targeting Cytochrome P450 2J2 (CYP2J2) and suppressing the Akt and mitogen-activated protein kinase (MAPK) pathways. In subcutaneous tumor-bearing mice treated with these MSCs Exo, tumor volume and weight were notably reduced [20]. Research has shown that exosomes from hMSCs overexpressing miR-375 inhibit U87 solute carrier family 31 member 1 (SLC31A1) expression in glioma cells, suppressing their proliferation, migration, and invasion, and promoting apoptosis. *In vivo* experiments have confirmed that these exosomes can inhibit the growth of xenografted tumors in nude mice [105]. Yan et al. discovered that bone mesenchymal stem cell (BMSC)-derived exosomes containing miR-512-5p can suppress U87 cell proliferation and induce cell cycle arrest by down-regulating Jagged1 (JAG1) expression. BMSC-Exo-miR-512-5p has been shown to inhibit the growth of mouse glioblastoma, thus prolonging survival [106]. Focused ultrasound (FUS) facilitates a transient, reversible, and localized opening of the BBB. Zhan et al. used hBMSCs-derived Exo as carriers for the tumor-suppressing gene miR-1208. Post-FUS exposure, an increased number of Exo carrying miR-1208 crossed the BBB, enhancing the uptake of miR-1208 by glioma cells U251 and U373. Mechanistically, miR-1208 suppresses methyltransferase-like 3(METTL3) expression, reducing the N6-methyladenosine (m6A) methylation level of Nucleoporin 214 (NUP214) mRNA. This reduction leads to decreased NUP214 expression and transforming growth factor-β (TGF-β) pathway activity, effectively suppressing tumor growth *in vivo* and *in vitro* [39].

Anti-angiogenesis

Vasculogenic mimicry (VM) provides an alternative microvascular circulation in tumors, independent of VEGF-driven angiogenesis. This process results in the formation of highly patterned vascular channels, particularly noted in gliomas, where VM structures are formed by differentiated tumor cells [107]. VM bypasses standard angiogenic mechanisms, a critical factor in sustaining the malignancy progression in GBM [108]. However, the molecular basis of VM formation remains only partially understood, leading to a lack of targeted therapies. In addressing this, Zhang et al. engineered hMSCs to produce exosomes with increased miR-29a-3p expression. This innovation inhibits glioma cell migration and VM formation, presenting a potential augmentation to current clinical treatments targeting angiogenesis [24]. In summary, modulating miRNA overexpression within exosomes can influence glioma characteristics, including drug resistance, growth, migration, apoptosis proficiency, and angiogenesis, offering promising avenues for effective glioma therapy (Figure 5B).

Engineering MSCs Exo for enhanced targeting

Strategies for the engineered modification of Exo

The limited therapeutic effectiveness of chemotherapy drugs largely stems from their systemic and non-targeted nature. In contrast, exosomes, while promising as drug delivery carriers, still face challenges in targeting efficiency. To address this, researchers have developed engineered exosomes for cell-specific targeting. By modifying their surface molecules, these exosomes gain cell and tissue specificity, allowing for the targeted delivery of specific tumor treatment molecules. The surface modification of exosomes can be achieved through genetic engineering or chemical modification. Genetic engineering involves fusing the gene sequences of guide proteins or peptides with those of select exosomal membrane proteins, thus displaying these guiding elements on the exosome surface. On the other hand, chemical modification employs conjugation reactions or lipid assembly to display a range of natural and synthetic ligands. However, the intricate nature of the exosome surface can sometimes reduce the efficiency of these reactions and potentially compromise the integrity and functionality of the carriers.

Currently, genetic engineering is a prevalent method for modifying exosomal surface proteins. In this process, ligands or targeting peptides are first fused with the genes of transmembrane proteins present on the exosome surface. Donor cells are then transfected with plasmids encoding these fusion proteins, leading to the production of exosomes that carry targeted ligands on their surface. A notable example is the work of Michelle E, who designed targeted peptide-Lamp2b fusion proteins with incorporated glycosylation sequences. These sequences protect the peptides from degradation and increase the overall expression of Lamp2b fusion proteins in both cells and exosomes. The glycosylation enhances the stability of these peptides, improving the targeting capabilities of the exosomes towards neuroblastoma cells [109].

Additionally, a technique known as “click chemistry” has been developed for covalently attaching modifications to the exosome surface. This method is advantageous due to its compatibility and rapid reaction rates. Nonetheless, careful control of several parameters, such as pressure, temperature, and osmotic pressure, is crucial during modification to prevent exosome rupture [110]. For instance, researchers have successfully targeted brain injury areas in cerebral ischemia models by attaching c(RGDyK) peptides to the exosome surface using bioorthogonal chemistry [111]. Another approach involves using non-covalent modifications to incorporate specific ligands or receptors onto exosome surfaces [110]. Kooijmans and colleagues, for example, combined epidermal growth factor receptor (EGFR)-specific nanobodies with phospholipid (DMPE)-polyethylene glycol derivatives to create nanobody-polyethylene glycol (PEG) micelles. These micelles can be integrated into exosome surfaces without altering their morphology, size distribution, or protein composition, significantly extending their circulation time and enhancing their tumor cell targeting capabilities [112].

The role of engineered modified MSCs Exo in glioma treatment

Rahmani et al. engineered MSCs Exo to merge with the anti-EGFRvIII antibody (ab139 scfv) linked to transmembrane protein Lamp 2b, and encapsulated both cytidine deaminase (CDA) and miR-34a, genes known for inducing apoptosis. The study revealed a significantly higher apoptosis induction rate in U87EGFRvIII cells compared to U87 cells, showcasing the selectivity of the engineered exosomes. Notably, after introducing CDA, miR-34a, and CDAmiR, the mortality rates in U87 cells were 6 %, 9 %, and 12 %, respectively, whereas for U87EGFRvIII cells, they increased to 13 %, 21 %, and 40 %. This indicates that bioengineered exosomes, carrying two gene therapy agents and targeting EGFRvIII antigen, substantially elevate apoptosis rates in GBM cells [32] (Figure 6A). Addressing TMZ-resistant GBM cells, which

exhibit high heme oxygenase-1 (HMOX-1) expression, researchers modified bone marrow mesenchymal stem cell exosomes (BMSC Exo) with HMOX-1 specific short peptides (HSSP) and encapsulated TMZ and STAT3 specific small interfering RNA (siSTAT3). *In vitro* and *in vivo* experiments showed that HSSP-BMSC Exo effectively targeted anti-TMZ GBM and, by silencing STAT3, modulated the STAT3-O6 methylguanine DNA methyltransferase (MGMT) pathway to induce apoptosis in TMZ resistant U251 (U251-TR) cells, thereby restoring drug sensitivity in these gliomas (Figure 6B). The allogeneic engineered HSSP-BMSC Exo thus emerges as an excellent carrier for TMZ-resistant GBM, characterized by outstanding biocompatibility, effective BBB penetration, prolonged blood circulation time, and specific targeting [33]. In conclusion, mesenchymal stem cell exosomes offer a versatile platform for loading anti-tumor drugs. By attaching protein encoding sequences or polypeptide links to their surface, their targeting ability can be significantly enhanced, optimizing the therapeutic impact. The therapeutic actions and mechanisms of MSCs Exo in glioma treatment are detailed in Table 2.

Surface modifications on exosomes enable the attachment of protein-coding sequences or peptides, which significantly enhances their targeting capabilities towards glioma cells. Additionally, these exosomes, when loaded with overexpressed gene therapy drugs or anti-tumor agents, can markedly induce apoptosis in glioma cells. This approach not only reduces chemotherapy resistance but also substantially improves the effectiveness of anti-tumor drugs.

Discussion and summary

There are still urgent issues to be resolved in the study of MSCs Exo in regulating glioma progression and its use as an engineered carrier for anti-tumor drugs. Current research primarily explores the impact of non-coding RNAs within MSCs Exo on glioma progression. However, future studies should broaden their scope to include the roles of other nucleic acids, lipids, proteins, and even mitochondria. Current studies on MSC therapy for glioma predominantly utilize BM-MSCs from humans or mice, and human umbilical cord-derived MSCs (Tables 1 and 2). One study reported that exosomes from adipose-derived MSCs do not inhibit glioma cell invasiveness [103], contrasting with another study that found exosomes from adipose sources can transfer miR-218 to breast cancer cells, reducing their invasiveness [113]. Thus, one significant challenge is identifying a consistent and suitable source of MSCs for drug delivery, as variations in MSC origins lead to exosomes with differing sizes, compositions, and functionalities. Additionally, the effect of cell passage number on exosome content remains unclear. While most research employs third or fourth generation MSCs for exosome collection, the impact of different generations on exosome contents and surface properties is not well understood. MSCs Exo retain certain characteristics of their parent cells, including tumor-specific targeting abilities. However, their specific molecular targeting mechanisms are not yet thoroughly studied. Future research should therefore delve into these molecular mechanisms to

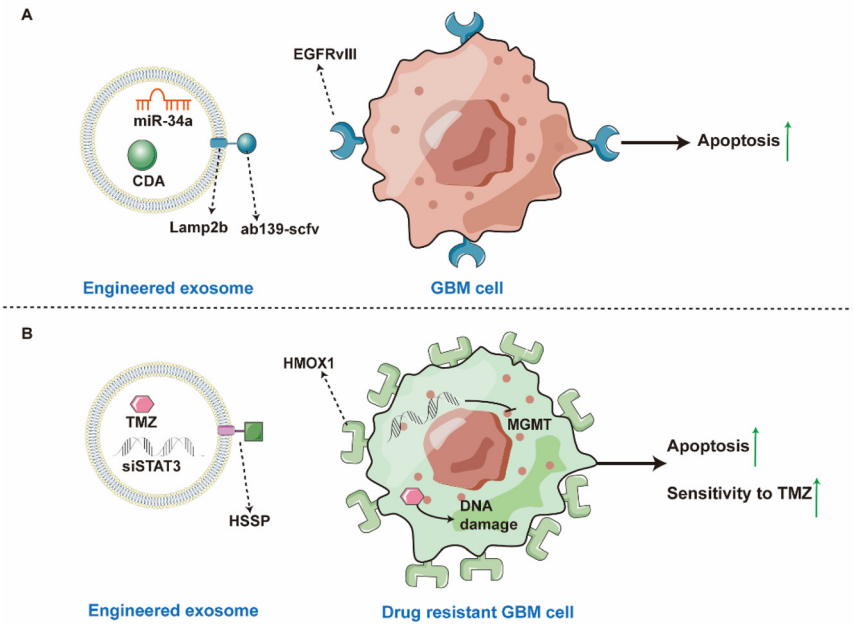

Figure 6: Engineered MSCs Exo in glioma treatment (the figure was drawn using AI and partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license).

Table 2: The therapeutic effects of MSCs Exo on glioma and related mechanisms ("N/A" indicates not mentioned or not applicable).

Exo/Evs type	Receptor cell	Passage number of cell	Loading drugs/ overexpressed molecules	Surface modification	Mechanisms/ targets	Function	Study model	Ref.
UC-MSC-EVs	U87	The third passage	Vincristine	N/A	N/A	Promote U87 cell apoptosis	*In vitro*	[103]
hEnMSCs-Exo	U87/ HUVECs	The third passage	Atorvastatin	N/A	N/A	Anti-tumor angiogenesis, migration and proliferation	*In vitro*	[25]
Wharton's jelly-MSCs-Exo	U87	Passage 3–4	miR-124	N/A	CDK6	Promote chemotherapy sensitivity of glioma cells	*In vitro*	[27]
hMSC-Exo	U87 T98G	N/A	anti-miR-9-Cy5	N/A	miR-9	Promote chemotherapy sensitivity of glioma cells	*In vitro*	[28]
TRAIL-MSCs-Exo	U87	N/A	miR-7	N/A	XIAP	Increase the sensitivity of TRAIL and synergize against tumors	*In vitro* and *in vivo* (U87-Balb/c nude mice *in situ* tumor formation)	[104]
hMSCs-Exo	GSCs	Passage 3–4	miR-124a	N/A	(FOX)A2	Reduce the viability and clonality of GSCs and prolong the survival of tumor-bearing mice	*In vitro* and *in vivo* (GSC267-Balb/c nude mice *in situ* tumor formation)	[21]
BMMSCs-Exo	9L	N/A	miR-146b	N/A	EGFR/NF-κB	Inhibit the growth of 9L cells *in vitro* and slow down tumor progression *in vivo*	*In vitro* and *in vivo* (9L-Fischer rat *in situ* tumor formation)	[22]
hMSCs-Exo	U87	N/A	miRNA-584-5P	N/A	CYP2J2-AKT/ MAPK	Inhibition of U87 invasion *in vitro* and slowing of tumor progression *in vivo*	*In vitro* and *in vivo* (U87-nude mice subcutaneous tumor formation)	[20]
hMSCs-Exo	U87	N/A	miR-375	N/A	SLC31 A1	*In vitro* suppression of U87's proliferation, migration, and invasion, promotion of apoptosis, and *in vivo* inhibition of xenograft tumor growth in nude mice	*In vitro* and *in vivo* (xenograft tumors in nude mice/specific tumorigenesis method unclear)	[105]
BMSC-Exo	U87	The third passage	miR-512-5p	N/A	JAG1	Inhibits glioblastoma cell proliferation and induces cell cycle arrest *in vitro*, inhibits glioblastoma growth and prolongs survival in mice *in vivo*	*In vitro* and *in vivo* (U87-nude mice *in situ* tumor formation)	[106]
hMSCs-Exo	U251/ U373	N/A	miR-1208	N/A	METTL3/ NUP214/TGF-β	Enhances hBMSCs-Exo-miR-1208 crossing the BBB; effectively inhibits tumor growth *in vivo* and *in vitro*	*In vitro* and *in vivo* (U251-female BALB/c nude mice *in situ* tumor formation)	[39]
hMSCs-Exo	U87/A172	N/A	miR-29a-3p	N/A	ROBO1	Inhibits glioma cell migration and VM formation	*In vitro* and *in vivo* (U87-Balb/c nude mice *in situ* tumor formation)	[24]
UCMSC-Exo	U87	N/A	CDA/miR-34a	Anti-EGFRvIII antibody	N/A	Promote U87 cell apoptosis	*In vitro*	[32]
BMSC-Exo	U251-TR	N/A	TMZ/siSTAT3	HSSP	STAT3-MGMT axis	Induces apoptosis in U251-TR cells and restores sensitivity of drug-resistant glioma to TMZ	*In vitro* and *in vivo* (U251-TR-Balb/c nude mice *in situ* tumor formation)	[33]

enhance targeting capabilities through genetic engineering. There is also a lack of research on how MSC exosomes affect different sources and types of glioma cells, an area that needs further exploration. Most existing research is confined to laboratory settings, presenting challenges in transitioning bioengineered MSCs Exo to clinical applications. The biological origins and mechanisms of exosomes are not fully understood, and numerous factors influence their formation, integration into target cells, profiling, and purification. These factors impact the modification and drug loading of exosomes. Another hurdle is the mass production of exosomes, requiring standardized processes for their separation, purification, drug loading, and modification. For MSCs in particular, the purification and cultivation conditions in the human body are stringent, adding to the complexity. In summary, the clinical application of MSCs Exo requires optimization in several areas, including large-scale production, separation, drug loading, and surface modification.

MSCs Exo, as a critical medium for intercellular communication, plays a pivotal role in glioma progression. While the exact function of MSCs Exo in glioma is still under debate, mainly due to variations in MSC origins and glioma progression stages, their potential as carriers for therapeutic drugs is broad and undeniable. MSCs not only possess common exosomal characteristics and unique advantages but also produce more exosomes compared to other cells, demonstrating strong tumor targeting capability. This capability could be further enhanced by surface modifications and alterations in content, improving the targeting and inhibition of glioma invasion. However, research on MSCs Exo is still in its infancy, with many unanswered questions. Our aim is to overcome these challenges soon and develop a more efficient and comprehensive glioma treatment strategy based on MSCs Exo.

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