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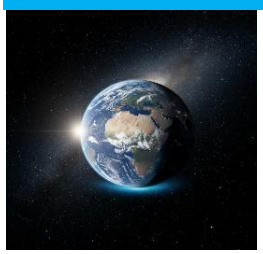

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Targeting glioblastoma. The potential of exosomes

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Abstract

Background: Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumor in adults and remains associated with a poor prognosis, with 5-year survival rates of only 4-7%. Even after surgery, radiotherapy and chemotherapy, the median survival rarely exceeds 12-16 months due to diffuse tumor infiltration, pronounced intratumoral heterogeneity and nearly inevitable recurrence. This therapeutic failure is caused in part by the blood-brain and blood-brain tumor barriers, which limit drug delivery and by glioma stem cells that sustain resistance and tumor regrowth.

Aim: The aim of this work is to evaluate exosomes as a promising drug delivery platform that can overcome these physiological barriers and improve treatment results for patients with glioblastoma.
Materials and methods: This analysis focuses on preclinical studies of engineered exosomes naturally secreted by various human cells. These nanoscale vesicles are studied as carriers for different types of therapeutic cargo, including chemotherapeutic drugs, nucleic acids and molecules that affect the immune system. The study examines how these vesicles are modified to target GBM cells directly.
Results: The results show that due to their endogenous origin, exosomes exhibit high biocompatibility, low immunogenicity, intrinsic targeting properties and the ability to cross the blood-brain barrier, offering advantages over conventional synthetic nanocarriers. Preclinical data demonstrate that engineered exosomes increase the amount of drug that accumulates inside the tumor, leading to higher cytotoxicity and a better response from the immune system. While there are still challenges regarding large-scale manufacturing and safety, exosomes represent a powerful tool for the future of precision-targeted therapy in glioblastoma.
Keywords: Glioblastoma, Exosomes, Extracellular vesicles, Drug delivery, Targeted therapy

Introduction

Glioblastoma (GBM) is the most frequent and aggressive primary brain cancer in adults. It is known for growing rapidly and spreading deep into the surrounding brain tissue, which makes it incredibly difficult to treat with standard therapies [1]. These types of brain tumors are among the most deadly cancers. Even though we have seen medical progress over the last several decades, five-year survival rates for GBM have only slightly improved from 4% to 7% [2]. Globally, the five-year survival rate generally stays between 4% and 17%, showing that the outlook for these patients remains very difficult regardless of where they are treated [3].
Today, the standard treatment for GBM involves removing as much of the tumor as safely possible through surgery, followed by radiation and chemotherapy with a drug called temozolomide. However, even this combined approach offers limited help and the cancer almost always comes back [4]. This happens largely because glioblastoma stem cells and the high variety of different cells within a single tumor (heterogeneity) drive resistance to treatment,

making a recurrence certain [5]. Because of these major obstacles, there is an urgent need for new drug delivery strategies that can cross the blood-brain barrier and handle the complex nature of the tumor.

Researchers are now looking closely at nanotherapies, because tiny carriers can better cross the blood-brain barrier, deliver drugs directly to the target and reduce side effects in the rest of the body [1]. In this field, exosomes have emerged as very promising "natural" delivery tools. Exosomes are tiny vesicles, usually between 30 and 200 nm that nearly all cells in our body release. They naturally carry proteins, lipids and different types of RNA to help cells communicate with each other [6]. In glioblastoma, these vesicles can naturally pass through the blood-brain barrier. They are currently gaining a lot of attention, both as "signals" that help us diagnose the tumor and as "delivery trucks" to carry anticancer drugs directly to the GBM tissue [7]. Because they come from living cells, exosomes are safe, circulate longer and have a natural ability to find their targets. This makes them an exciting new generation of drug delivery systems that could finally overcome the limits of current therapies and support more precise treatment for glioblastoma [8].

Glioblastoma - biological and therapeutic challenges

GBM is the most common primary brain cancer in adults, characterized by its aggressive nature. In the WHO classification, it is a grade IV diffuse astrocytic glioma, a label that reflects its highly invasive nature and poor prognosis. Even with the best available treatment, the outlook remains difficult. Glioblastoma accounts for a large portion of malignant brain tumors in adults and the five-year survival rate is only about 7% [2]. The current "standard of care" - which involves resection of as much of the tumor as possible through surgery, followed by radiation and chemotherapy with temozolomide - usually only extends survival to 12-16 months. Ten-year survival is extremely rare, staying below 1% [2,4,9]. The main reason we cannot achieve long-term control is GBM’s biology. It infiltrates the surrounding healthy brain tissue early on and spreads micrometastases throughout the central nervous system. Furthermore, the blood-brain barrier (BBB) and the blood-brain tumor barrier (BTB) act as physical shields, preventing drugs from reaching the tumor at effective doses. Because of these anatomical obstacles, GBM is the perfect example of a disease in which new strategies, including nanocarriers and exosome-based delivery systems, are urgently needed [10,11,12,13].

One of the most striking features of glioblastoma is its extreme heterogeneity. This means the tumor is not a uniform mass, it is a complex mosaic that varies genetically and metabolically between different patients and even within different areas of the same tumor. Genomic studies have identified specific molecular subtypes, such as the mesenchymal subtype. Compared to other transcriptional subtypes, the mesenchymal class is notably more aggressive. Its resistance to multiple therapies is driven by high levels of hypoxia and inflammation, which results in a less favorable prognosis [5,9,14].

Single-cell sequencing has shown that within a single tumor, cells can exist in different states including astrocyte-like, oligodendrocyte-like, neural progenitor-like and mesenchymal-like populations. These cell populations shift over time, especially when under the pressure of treatment. This diversity is spatially organized. For example, specific cells hide in perivascular or hypoxic niches, creating local environments that differ across tumor zones and protect them from the immune system. This complexity is why a drug might kill one part of the tumor while another part continues to grow [5,9,14].

Within this diverse ecosystem, Glioma Stem Cells (GSCs) act as the primary drivers of the tumor. These cells can self-renew and differentiate into various cell types, effectively "reseeding" the tumor after treatment [5]. GSCs possess self-renewal capacity, multipotent differentiation potential and heightened ability to evade immune surveillance and are often enriched after chemoradiotherapy, because they are naturally resistant to DNA damage [14,15]. Recent research using single-cell and lineage-tracing methods shows that different GSC lines produce a wide variety of cancer cell types. Together, these cells create a highly diverse tumor environment and actively control how immune cells enter and function within the tissue. Because even a very small number of GSCs can restart tumor growth (reseed), successfully curing the disease depends on specifically destroying these cells [5,15,16]. Consequently, scientists are developing new ways to target the specific signals, environments and metabolism of GSCs. These strategies often use advanced drug delivery tools and immunotherapies. For example, researchers are testing engineered Natural Killer (NK) cells and NK-cell-derived exosomes to reach and eliminate GSCs within the protective areas where they hide [13,16].

The BBB is a highly regulated filter made of endothelial cells, pericytes and astrocytic endfeet that protects the brain from harmful substances in the blood [10,11]. In glioblastoma, the barrier is damaged in the tumor’s center, leading to a compromised blood-brain tumor barrier with varied permeability. At the same time, the barrier remains mostly functional at the outer margins,

where the tumor cells that cause recurrence are hidden. [11]. This creates duality, that drugs might reach the center of the tumor, but fail to reach the invading cells at the margins. Interestingly, successful chemotherapy can sometimes restore the BBB, paradoxically making it harder for drugs to get through in later cycles [10]. To overcome this, science is moving toward non-invasive strategies like exosomes. Because exosomes are endogenous, they are small and can be engineered with surface ligands to cross the BBB naturally, delivering chemotherapeutics, genes or immunomodulators directly to the tumor and stem cell compartments [7,12,13].

Even with a combination of different treatments, glioblastoma is highly resistant to chemotherapy and radiation, which means the tumor almost always recurs. [4,9]. GBM cells and GSCs, have highly active DNA damage response pathways. They can stay in a quiescent or slow-growing state, which makes them less sensitive to radiation and drugs like temozolomide that target fast-growing cells [14,15]. Furthermore, the transition to a mesenchymal-like state, often triggered by therapy, makes the tumor more invasive and helps it build an immunosuppressive microenvironment. The brain is already an immunologically "cold" environment and the tumor makes it even harder for the immune system to work by releasing specific factors and extracellular vesicles [5,13]. To win this fight, we need combination strategies that target both the main tumor mass and the GSCs, using innovative platforms like exosomes to reprogram the tumor environment and increase the precision of our treatments [7,11,13].

Isolation of exosomes

Exosomes are nanovesicles (30-200 nm) released by almost all types of cells. They are increasingly recognized as powerful tools for both diagnosing and treating cancers like glioblastoma. To use them in a clinical setting, we need reliable, high-quality methods to isolate them from biological sources like blood, tissues or cell cultures. Standard methods like ultracentrifugation and precipitation are still common, but they are slow and demanding. These techniques often lead to inconsistent results and lower purity, especially when trying to produce exosomes on a larger scale. Furthermore, these older techniques can sometimes damage the vesicles or result in low purity. Studies comparing various techniques confirm that dual-method approaches, most often SEC paired with ultrafiltration, are superior to single techniques. By

improving both yield and purity, these combined processes have become the standard recommendation for manufacturing therapeutic exosomes. [17,18,19].

Recent technological breakthroughs, such as microfluidic and immunoaffinity-based platforms, use the physical and chemical properties of exosomes, like their size, charge, or surface markers for isolation. These systems provide a faster and gentler way to collect exosomes while keeping them intact. Because they offer more consistent results and lose less material, these platforms are ideal for clinical applications and the development of liquid biopsies [17,18,20]. At the same time, industrial-scale methods like tangential flow filtration (TFF) and bioreactors are being developed. These processes follow Good Manufacturing Practice (GMP) standards, which are essential for producing enough exosomes to be used in human clinical trials [21,22].

For glioblastoma patients, exosomes are a major breakthrough because they can naturally cross the blood-brain barrier and the blood-brain tumor barrier. They can protect therapeutic cargo, while remaining safe for the patient's immune system. These vesicles can be loaded with chemotherapy or genes and sent directly to glioblastoma cells, which increases the drug's effectiveness in the brain while reducing side effects in the rest of the body. The way we isolate these exosomes is critical. To successfully target the brain, we must ensure the vesicles remain structurally intact and that their surface ligands are preserved. These surface proteins provide the essential targeting cues needed for the exosomes to cross the blood-brain barrier and find the tumor cells. If the isolation process damages these proteins, the exosomes will lose their ability to find and enter the tumor [8,10,23]. Although this technology is still mostly in the preclinical phase, improvements in production and quality control are quickly bringing us closer to using exosomes as a next-generation treatment for glioblastoma [17,18,20,22].

Advantages of exosomes over synthetic nanocarriers

Exosomes vs. Liposomes: Structural and Functional Differences

Exosomes are natural, cell-derived vesicles that offer significant biological advantages over synthetic platforms like liposomes or polymeric nanoparticles. As endogenous messengers, they carry a specific repertoire of proteins, lipids and nucleic acids that mirror their parent cells, ensuring high biocompatibility and seamless integration into intercellular communication pathways [24,25]. This natural origin provides superior immune tolerance, unlike synthetic systems that often require chemical modifications like PEGylation to hide from the immune system, exosomes naturally avoid rapid clearance and inflammatory reactions [24,26].

Furthermore, exosomes possess intrinsic targeting capabilities. Their surface proteins and ligands allow for precise tissue and cellular tropism - a natural homing ability that liposomes can only achieve through complex artificial engineering [11,25]. In clinical applications, one of their most critical features is the capacity to cross biological barriers, most notably the blood-brain barrier. This allows them to deliver therapeutic cargo to the central nervous system, a task that remains difficult and inconsistent for conventional synthetic carriers [11,24]. Their unique membrane composition also protects delicate cargo, such as nucleic acids, from degradation and ensures efficient delivery directly into the target cell's cytoplasm. By combining these natural stealth properties with superior barrier penetration, exosome-based therapies can achieve higher efficacy at lower doses, ultimately reducing off-target toxicity [24,25,26]. While liposomes are currently easier to manufacture at scale, the sophisticated biological machinery and physiological harmony of exosomes position them as a more effective platform for next-generation precision medicine in cancer and neurological diseases [11,24].

Biocompatibility and low immunogenicity

Because exosomes are naturally produced by cells, they possess an inherent biological compatibility that synthetic carriers struggle to replicate. These vesicles closely resemble the body’s own cell membranes, which allows them to deliver therapeutic cargo with high efficiency while triggering minimal toxicity or unwanted immune responses [24]. This endogenous origin provides a much safer clinical profile than many lipid- or polymer-based nanoparticles. While synthetic systems often require complex surface modifications to avoid being detected and killed by the immune cells, exosomes naturally bypass these defenses because the body recognizes them as its own [25,26]. In the specific context of glioblastoma, this natural harmony is vital - it enables the low-toxicity delivery of potent immunotherapies while significantly reducing off-target immune effects. This makes exosomes an exceptionally attractive platform for cases where repeated or systemic administration is necessary to control tumor growth [11,13].

Instrinsic targeting and homing effect

Exosomes have a natural ability to find and enter specific cells because they inherit membrane proteins and ligands from their cells of origin. This endogenous tropism allows them to deliver their cargo much more precisely than synthetic nanocarriers, which usually require artificial

molecules to be attached to their surface to find a target [7]. This natural homing is especially valuable in treating glioblastoma. Unlike most synthetic nanoparticles, which struggle to pass the blood-brain barrier and often require invasive procedures to reach the brain, exosomes can cross both the BBB and the BTB on their own. This allows for the non-invasive delivery of drugs, genes and diagnostic tools directly into the central nervous system [10]. Ultimately, the main advantage of exosomes is that they combine the ability to cross the BBB with cell-specific targeting in one physiological package. This makes them a safer, less toxic and potentially more effective platform for treating brain tumors and other neurological diseases [7,10].

Loading strategies

Drug loading into exosomes for glioblastoma therapy is generally classified into passive and active strategies. These methods differ in their loading efficiency, technical difficulty and how much they affect the structural integrity of the vesicles.

Passive loading usually depends on the natural diffusion of small, hydrophobic drugs across the exosome membrane. This is often achieved through simple co-incubation or by using pH and ion gradients. Research on extracellular vesicles (EVs) shows that these methods are simple and gentle. They help maintain the exosome's size and the original surface proteins that are necessary for the exosome to reach the brain and the tumor [27]. A clear example in glioblastoma research is the alkaline passive loading of doxorubicin into neural stem cell-derived EVs. This method achieved high loading efficiency and killed glioma cells effectively. These EVs also crossed the BBB more efficiently than the drug alone, proving that passively loaded EVs are effective carriers for CNS treatments [28]. However, passive methods often suffer from low efficiency and are difficult to scale up for mass production. This is especially true for hydrophilic drugs and large molecules, which remain a major challenge for using these methods in high-dose clinical cancer treatments [29].

Active loading strategies provide more control over the type and amount of cargo. This is done either by forcing drugs into isolated exosomes or by genetically modifying the parent cells so they release vesicles already containing the therapeutic molecules. Direct active methods include electroporation, sonication and freeze-thaw cycles. These techniques significantly improve the packaging of siRNA, mRNA, proteins and polar drugs. However, they can damage the exosome membrane, cause the vesicles to clump together or change surface markers that are vital for targeting glioblastoma cells [30].

Genetic engineering of producer cells is particularly effective for RNA-based GBM therapies, as these EVs can safely carry therapeutic RNA across the BBB [31]. A notable example for glioblastoma involves a microfluidic electroporation system that created EVs loaded with IFN-γ mRNA. These EVs also expressed CD64, which allowed for antibody attachment. The resulting immunogenic EVs targeted GBM cells specifically and triggered strong anti-tumor responses in vivo, even in tumors that were resistant to standard immunotherapy [32]. Active loading also integrates well with surface engineering to improve GBM targeting. For example, RGD-engineered EVs showed 40% higher accumulation in glioblastoma cells and made the delivered doxorubicin and siRNA more toxic to the tumor compared to standard EVs [33].

In conclusion, passive loading is most suitable for small, lipophilic drugs when maintaining the exosome's natural structure is the priority. In contrast, active loading is better for high-density RNA or protein delivery and for precise targeting. Together, these two strategies offer different ways to use exosomes effectively against glioblastoma [34].

Therapeutic cargo in glioblastoma targeting

Exosomes are tiny extracellular vesicles (EVs) with lipid-protein membranes that mirror the biochemical composition of their parent cells. This allows them to deliver biological cargo to specific locations and change the function of target glioblastoma cells [35]. Because they remain stable in body fluids, do not trigger a strong immune response and can cross physiological barriers, they are excellent candidates for targeted GBM therapy. These features are particularly useful given the high level of tumor heterogeneity and treatment resistance found in glioblastoma [5,36].

When used to carry chemotherapy, exosomes help drugs pass through the blood-brain barrier and the blood-tumor barrier, leading to a higher concentration of the drug within the tumor. For

example, EVs sourced from human neural stem cells and loaded with doxorubicin, using a passive alkaline method, were highly toxic to glioma cells. These EVs crossed the BBB more effectively than doxorubicin alone, showing that the performance of the carrier depends heavily on both the cell source and the loading method [28].

Furthermore, doxorubicin nanoparticles coated with endothelial exosomes have shown the ability to penetrate the BBB, trigger apoptosis and cause immunogenic cell death. In animal studies, this method improved survival. It proved that exosomal coatings can effectively deliver drugs while also helping the immune system kill the tumor [37].

As nucleic acid carriers, exosomes take advantage of their natural ability to transport RNA to regulate oncogenic signaling and target the glioblastoma stem-cell population. Current technology allows for the efficient packaging of miRNA, siRNA, mRNA and CRISPR/Cas components. This is achieved either by genetically modifying the donor cells or by using active loading techniques like electroporation and dimerization [38,39. For example, RGD-engineered EVs carrying siRNA successfully lowered GAPDH gene expression in GBM cells. This proves that targeted EVs can effectively deliver RNA interference (RNAi) to this specific type of tumor [33]. Additionally, microfluidic electroporation has enabled the large-scale production of small EVs loaded with IFN-γ mRNA that also overexpress CD64. These vesicles can dock specific antibodies, such as anti-CD71, to target glioblastoma cells more precisely. This platform has shown strong antitumor activity in vivo and represents a versatile tool for delivering mRNA or CRISPR components to resistant GBM clones [32].

Immunomodulatory cargos delivered via exosomes aim to reprogram the highly immunosuppressive microenvironment of glioblastoma. This environment is maintained by treatment-resistant stem cells and complex interactions between the tumor and the immune system [5]. Exosome-based delivery systems are now being designed to increase the tumor's immunogenicity and reverse local immunosuppression, which helps improve the effectiveness of standard immunotherapies [13].

Limitations and challenges

Scalability is a major obstacle for exosome-based glioblastoma therapies. Traditional laboratory methods, such as ultracentrifugation, produce only small amounts of vesicles, are labor-intensive and difficult to use for mass production. New solutions are being developed, including bioreactor-based expansion and the use of tangential flow filtration (TFF) and size-exclusion

chromatography. These techniques aim to enable Good Manufacturing Practice (GMP)-compliant production. However, these technologies still require significant technical expertise and investment before they can be used routinely in clinics [21,22,30].

Standardization is also difficult because exosomes vary depending on their source, how they are isolated and how they are engineered. This leads to batch-to-batch variability in their size, cargo and biological activity. The lack of universal quality-control standards and validated potency assays makes it hard to compare results across different studies. This lack of clear regulatory guidelines complicates the process of getting these therapies approved for glioblastoma treatment [22,30,36].

Safety concerns include the risk of an immune response, off-target biodistribution and toxicity from the exosome cargo or impurities. A major clinical issue is the rapid clearance of exosomes by the mononuclear phagocyte system (MPS), which often requires the use of camouflage to hide the vesicles from the immune system. Before clinical use, rigorous testing is needed to evaluate how these vesicles move through the body and whether they cause genetic or immune toxicity [30,35,40].

A critical risk is that exosomes may accidentally promote cancer growth. This is because vesicles derived from glioblastoma or surrounding stromal cells can naturally encourage tumor cell division, invasion and angiogenesis. Using exosomes that are poorly characterized or derived from tumor cells might unintentionally help the cancer spread or develop resistance to therapy. Therefore, it is essential to carefully select the parental cells, remove any cancer-promoting cargo and perform strict functional testing to ensure the therapy does not support tumor growth [30,35,36].

Conclusion and future perspectives

Glioblastoma remains a challenging malignancy because the blood-brain and blood-brain tumor barriers and high intratumoral heterogeneity limit the effectiveness of the standard Stupp protocol chemoradiotherapy. Exosomes offer a solution by combining natural biocompatibility and BBB penetration with the ability to deliver chemotherapy, nucleic acids and immunomodulators through targeted surface engineering [10,13,36]. Studies confirm that these vesicles improve drug accumulation in the brain and can carry complex cargo, such as IFN-γ mRNA, to trigger potent anti-tumor immune responses [16,32,37]. However, clinical translation is still hindered by manufacturing difficulties, low loading efficiency and the need

for long-term safety data within the human brain environment [10,13,36]. Future efforts must focus on clinical-grade production and subtype specific targeting to transform exosomes from experimental tools into realistic precision therapies for GBM [10,13,36].

Disclosure

Author's contribution
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Formal analysis – Patryk Bachurski, Maja Międlar
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