Development of Targeted Tumor-Mimicking Nanoparticles for Chemotherapeutic Drug Delivery and Modulation of the Glioblastoma Immune Microenvironment

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Background of research

Glioblastoma (GBM) is a highly invasive and malignant brain tumor that accounts for approximately 49% of malignant brain tumors[1]. Current standard treatments for glioblastoma include surgical resection, chemotherapy (such as temozolomide), radiotherapy, and bevacizumab[2-4]. However, despite improvements in short-term survival rates, the overall prognosis for glioblastoma remains poor, with a median survival of less than 2 years and a high recurrence rate[5].

The treatment of glioblastoma encounters substantial challenges attributed to the distinctive characteristics of the tumor microenvironment (TME), which encompass the presence of the blood-brain barrier (BBB) and the immunosuppressive nature of glioblastoma[6]. These factors have prompted extensive research and development of novel treatment strategies. Current therapies for glioblastoma, such as chemotherapy and radiation therapy, have inherent immunosuppressive effects[3]. Immunotherapy has emerged as a promising direction currently under clinical investigation. Although immune checkpoint inhibitors have shown promising therapeutic activity in clinical settings, they have not demonstrated significant efficacy in recurrent glioblastoma[7]. Vaccine therapy is constrained by the selection of antigens and the heterogeneous expression of tumor antigens. Additionally, chimeric antigen receptor (CAR) T-cell therapy for glioblastoma relies on identifying tumor-specific antigens, which poses significant limitations[3]. Therefore, using biomarker-enrichment immunotherapy and precision oncology approaches represents an urgent challenge in developing effective targeted therapies for glioblastoma[3, 8]. Immunotherapy-based combination treatment strategies have the potential to overcome immune suppression within the glioblastoma TME, converting "cold" tumors into "hot" tumors and thereby enhancing treatment efficacy.

CXCR4 is prominently expressed on the surface of diverse cell types, including stem cells, immune cells, and cancer cells. The CXCR4/CXCL12 axis is crucial in hematopoiesis, embryonic development, immune response, and cancer metastasis[9]. Accumulation of antigen-specific $CD8^+$ T cells within tumors is a prerequisite for effective immune therapy. By inhibiting CXCR4, the egress of T cells from TME can be restricted, enhancing the retention of T cells within the TME, and thereby improving the efficacy of immunotherapy[10]. AMD3100, an FDA-approved small molecule antagonist targeting the CXCR4 receptor, has been utilized for peripheral blood stem cell transplantation[11]. It has been shown to induce rapid accumulation of T cells within cancer cells and has been validated in a mouse model of pancreatic cancer, demonstrating that sustained CXCR4 inhibition increases T cell infiltration into tumors[12]. Furthermore, CXCR4 is widely expressed in cancer cells and is involved in tumor progression, angiogenesis, metastasis, and survival[13]. In GBM, activation of the CXCR4/CXCL12 axis promotes tumor cell proliferation and induces tumor angiogenesis by stimulating the release of vascular endothelial growth factor (VEGF) from glioblastoma stem cells (GSCs), thereby enhancing tumor growth.[14]. CXCR4 antagonists exhibit anti-tumor activity in various malignancies and can be used for the treatment of glioblastoma[15], hepatocellular carcinoma[16], pancreatic cancer, and colorectal cancer[17] , etc. Despite the widespread clinical application of AMD3100 as a CXCR4 antagonist, its use is limited due to suboptimal pharmacokinetics and long-term toxicity associated with non-specific targeting[16]. Moreover, its application in glioblastoma is restricted by the BBB.

In recent years, tumor cell membrane-coated nanoparticles have emerged as a promising therapeutic platform for cancer treatment[18]. The homotypic binding of tumor cell membranes allows these nanoparticles to serve as targeted carriers, while cell adhesion-related proteins enable specific interactions with tumor cells, facilitating the adhesion and internalization of nanoparticles into the target cells[19]. These interactions enhance the capacity for targeted treatment of glioblastoma, enabling efficient delivery of therapeutic drugs to the tumor site and thereby improving treatment efficacy while minimizing potential harm to the organism[20]. Moreover, the presence of abundant tumor-associated antigens (TAAs) within the cell membrane offers opportunities to promote anti-tumor immune responses and enhance the effectiveness of immunotherapy[19].

In this study, as shown in Fig. 1, we propose utilizing glioblastoma cell membrane-coated poly (lactic-co-glycolic acid) (PLGA) nanoparticles (CM-NPs) to selectively deliver the chemotherapeutic drug rapamycin (RAPA) to the TME. RAPA inhibits the mTOR pathway, suppressing angiogenesis and inhibiting primary and metastatic tumor growth[21]. To counteract the immunosuppressive TME and T cell exclusion caused by chemotherapy, the nanoparticles will be loaded with the CXCR4 antagonist AMD3100. This approach aims to enhance the infiltration of cytotoxic CD8+ T cells into GBM, inhibit tumor angiogenesis, and effectively suppress tumor progression. Additionally, the cell membrane coating will present TAAs, promoting immune responses.

In our preliminary experiments, we successfully coated the cell membrane of glioblastoma cells onto PLGA nanoparticles. Dynamic light scattering (DLS) measurements were conducted to determine the particle sizes and surface zeta potentials of the nanoparticles. The results, as shown in Fig. 2a, indicated that the sizes of the uncoated nanoparticles (NPs) and cell membrane-coated nanoparticles (CM-NPs) were 157.2 ± 2.393 nm and 165.3 ± 4.825 nm, respectively. The surface zeta potentials of NPs and CM-NPs were found to be -49.4 ± 6.180 mV and -35.43 ± 2.984 mV, respectively. Furthermore, long-term storage capability of CM-NPs was evaluated by monitoring their size stability in phosphate-buffered saline (PBS) over a period of 45 days, as presented in Fig. 2b. The results demonstrated that CM-NPs maintained a stable size throughout the storage period, indicating their potential for long-term storage. Additionally, the detachment of the coated membrane was assessed by washing CM-NPs with fetal bovine serum (FBS), and no detachment was observed, as depicted in Fig. 2e. The co-localization coefficient was calculated as 0.5812 (in PBS) and 0.6512 (in FBS), validating the retention of fluorescent markers and the stability of CM-NPs. Moreover, transmission electron microscopy (TEM) images and scanning electron microscopy (SEM) demonstrated the relatively uniform particle sizes and the core-shell structure of CM-NPs, as shown in Fig. 2c and Fig. 2d. These results confirm the successful construction of cell membrane-coated nanoparticles. Based on these preliminary experimental results, we have established the feasibility and effectiveness of coating PLGA nanoparticles with GBM cell membranes. These findings provide a strong foundation for the proposed research, which aims to utilize these CM-NPs for targeted drug delivery and immunotherapy in glioblastoma treatment.

The ability of the glioblastoma cell membrane-coated nanoparticles (CM-NPs) to target and penetrate the BBB is crucial for effective drug delivery to the brain tumor site. We utilized a Transwell system to construct an in vitro BBB model to validate the targeting ability of the glioblastoma cell membrane, as shown in Fig. 3a. After incubating the nanoparticles with cells for 4 hours, we performed observations using confocal laser scanning microscopy (CLSM). The results clearly demonstrated that the nanoparticles coated with glioblastoma cell membranes exhibit a higher degree of cellular uptake by the glioblastoma cells in the lower chamber of the Transwell compared to the uncoated PLGA core (Fig. 3b). Furthermore, the results from flow cytometry analysis also demonstrated a significant increase in the accumulation of CM-NPs within glioblastoma cells U87-MG in the lower chamber compared to NPs at different time points of 1 hour, 2 hours, 4 hours, and 6 hours of incubation (Fig. 3c). In addition, we conducted preliminary investigations into the mechanisms underlying the internalization of CM-NPs by brain microvascular endothelial cells (BMECs) bend.3, as shown in Fig.3d. The bend.3 cells were pre-treated with dynasore, blebbistatin, chlorpromazine, or cytochalasin D, and the internalization of CM-NPs by the endothelial cells was studied using CLSM. The results revealed a significant inhibitory effect on the internalization process when cells were pre-treated with dynasore and cytochalasin D. This indicates that the internalization of cell membrane-coated nanoparticles is influenced by dynamin and the cellular cytoskeleton.

Based on current research findings, it is evident that the GBM cell membrane can confer in vitro targeting ability to poly (lactic-co-glycolic acid) (PLGA) nanoparticles, effectively facilitating their internalization. However, to assess the in vivo targeting efficacy and therapeutic effects of drug loaded nanoparticles on glioblastoma (GBM), as well as their modulation of the immune microenvironment,

it is necessary to conduct studies using animal models. Evaluation of the therapeutic efficacy of the glioblastoma cell membrane coated nanoparticles loaded with RAPA and AMD3100 (CM-NPs@AMD3100/RAPA), a nanomedicine, can be achieved by assessing biodistribution of nanoparticles within tumor tissue, drug release kinetics, changes in tumor volume, and activity of immune cells.

Figures

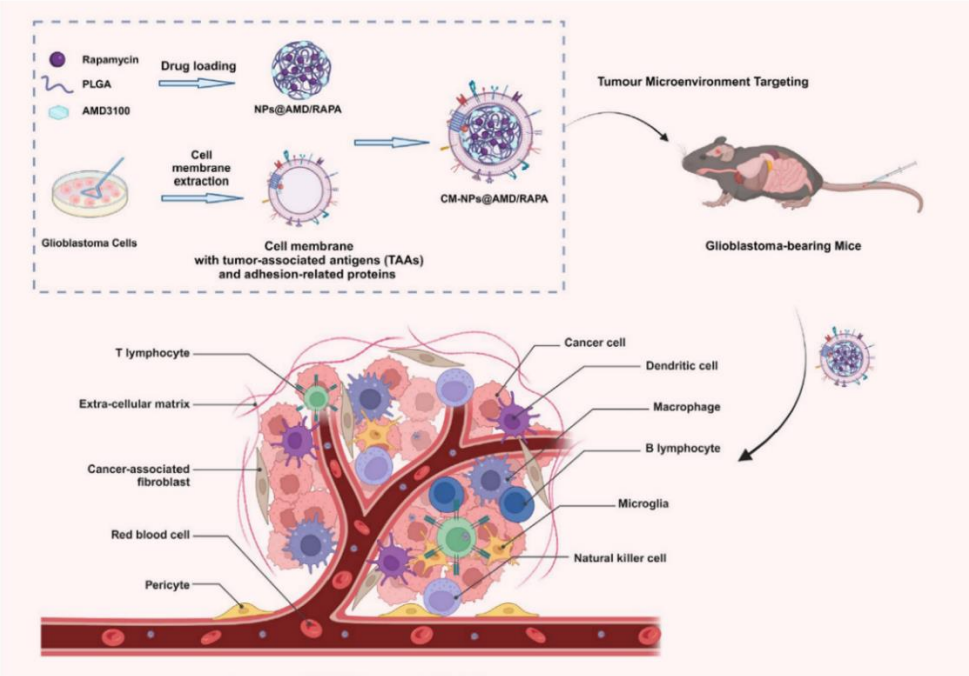

Figure 1. Schematic representation of the research plan. (a) Synthesis of PLGA nanoparticles loaded with rapamycin and AMD3100, coated with GBM cell membrane. (b) Injection of nanoparticles into mice to modulate the TME.

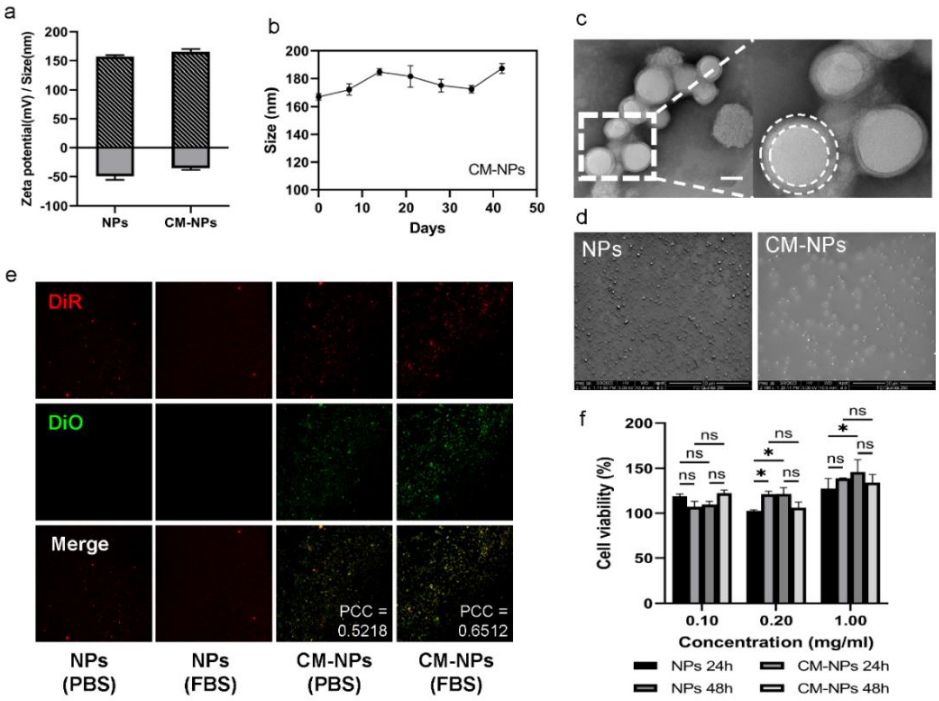

Figure 2. (a) Particle size and zeta potential of NPs and CM-NPs measured by DLS. b Long-term size stability of CM-NPs in PBS. (c) Transmission electron micrographs (TEM) analysis of CM-NPs. Scale bars = 100 nm. (d) SEM image of NPs and CM-NPs (e) Stability of NPs and CM-NPs characterized by CLSM after storage for 7 days in PBS and FBS. PLGA cores loaded with DiR (red) and cancer cell membranes labeled with DiO (green). Scale bar = 100 μm. (f) Cytotoxicity of 0.1, 0.2, and 1mg/mL NPs, CM-NPs at 24h and 48h.

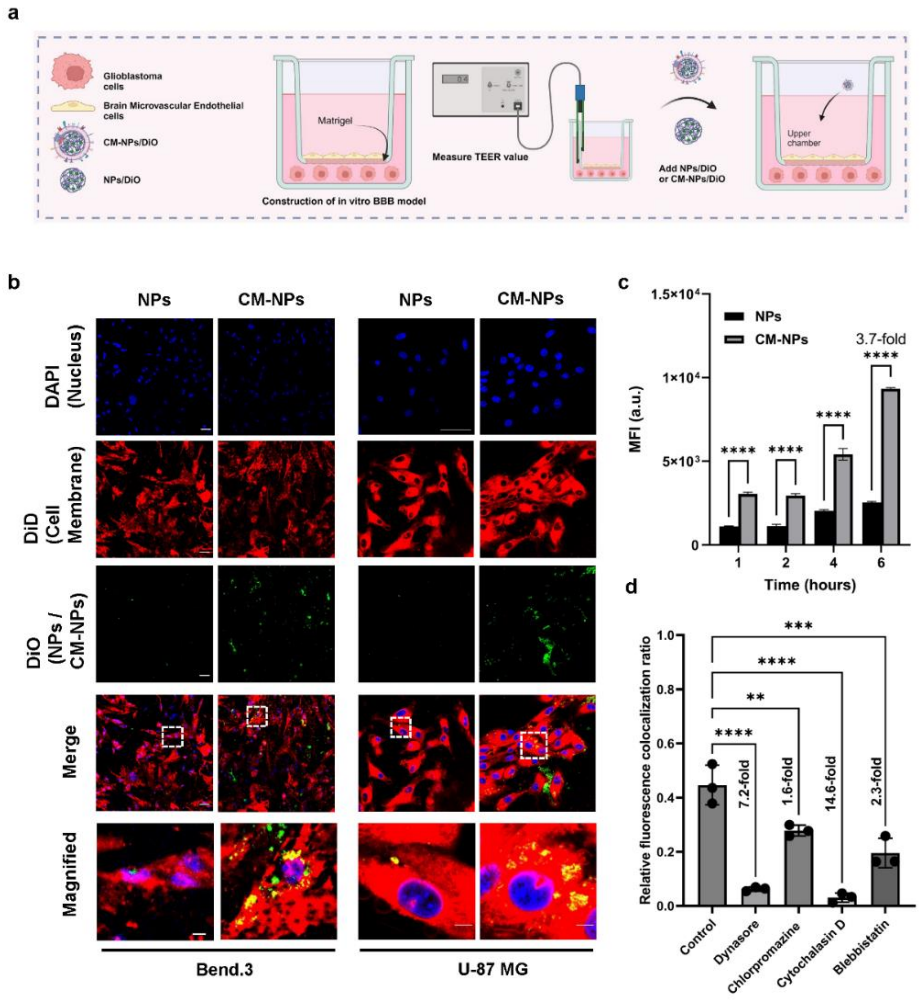

Figure 3. (a) Schematic representation of the construction of an in vitro BBB model. (b) Representative CLSM images of the cell internalization of NPs and CM-NPs crossing Transwell. Scale bar = 50 μm (main image), 10 μm (magnified image). (c) Internalization of NPs and CM-NPs by U-87 MG cells in the lower chamber of the Transwell system was quantified through flow cytometry analysis at 1 h, 2 h, 4 h, and 6 h time points. Statistical analysis was performed with two-way ANOVA. The data are shown as mean ± SD (n = 3). (d) The influence of inhibitors on cellular uptake of NPs and CM-NPs in bend.3 for 4h as detected by CLSM. The Pearson fluorescence colocalization coefficient of CM-NPs and cell membrane was quantitatively analyzed using ImageJ. Statistical analysis was performed with one-way ANOVA. The data are shown as mean ± SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

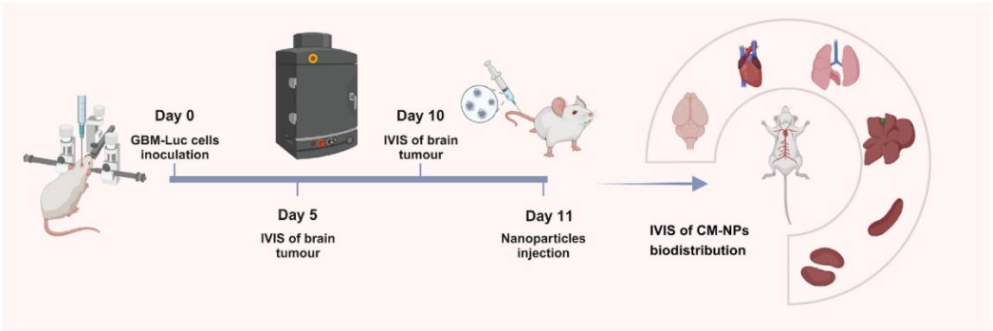

Figure 4. Schematic representation of the construction of a glioblastoma animal model and nanoparticle biodistribution study.

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