Pre-clinical Development of Nanoparticles and Nanogels for Drug and Gene Delivery to Glioblastoma
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Chapter 5

Summary and future perspectives
Summary

Glioblastoma multiforme is one of the most common malignant gliomas in the central nervous system, accounting for 82% of malignant glioma cases. Despite extensive surgical resection and adjuvant therapy, the median survival of GBM patients is only about 12-18 months, and the overall 5-year survival rate is less than 10%, with just a few patients surviving for 10 years or longer. This situation has remained unchanged for more than 60 years. Also, many patients are not recommended for surgery due to an inoperable location of the tumor or poor physical performance of the patient.

Therefore, while studying the complex mechanism of GBM including growth, progression and invasion and chemotherapeutics-resistance process, researchers also devote themselves to exploiting other treatment strategies to improve the survival rate of GBM, such as gene therapy, immunotherapy, phototherapy and thermotherapy, etc. A large number of nanomaterials have been used to try and solve the problem of therapeutic agents, e.g., short circulating half-life, difficulty in accumulating in tumor area, controlling drug release, and overcoming the barriers of GBM. These nanomaterials showed enhanced anti-tumor effects in animal models. At present, only a few malignant Glioma therapy options have been approved by the US Food and Drug Administration (FDA), including DNA alkylating agent temozolomide and nitrosoureas alkylating agents (carmustine and lomustine), carboplatin as well as monoclonal antibody bevacizumab that targets angiogenesis.

Therefore, it is necessary and promising to study and screen more drugs for adjuvant treatment of GBM. Although the mechanism is not completely clear, many natural compounds have shown anti-tumor activity in in vitro cell experiments or in vivo animal models. In this thesis, curcumin, a natural phenolic compound extracted from the curcuma longa rhizomes, was selected as the research drug (Chapter 2). We used amphiphilic zein protein, which can self-assemble into nanoparticles, to incorporate curcumin, thereby overcoming the disadvantages like poor water solubility and low bioavailability of curcumin. In order to make the nanoparticles pass through the blood-brain barrier, the nanoparticles were functionalized with the ganglioside GM1-binding G23 peptide that has been shown to mediate BBB crossing of polymersomes, RNA-binding proteins, and iron oxide nanoparticles. We showed that G23-functionalized zein nanoparticles efficiently traversed an in vitro BBB model, and stimulated 3D tumor spheroid penetration. Further, it was observed that the curcumin-loaded zein nanoparticles markedly inhibited proliferation and migration and induced cell death in liquid and soft agar models of C6 glioma cells. To investigate the biodistribution of zein nanoparticles in the body, we intravenously injected the nanoparticles in zebrafish and observed that G23 functionalized nanoparticles exhibited the ability to circulate in the blood, showing their potential as a delivery platform for drugs to treat GBM.

Single chemotherapy usually leads to drug-resistance and tumor cell tolerance after a certain period, and ultimately leads to tumor recurrence and metastasis. To overcome these problems, researchers have adopted a combination therapy strategy by combining multiple drugs with diverse mechanisms or combining multiple therapeutic strategies, such as combining chemotherapy with radiotherapy, combining chemotherapy with phototherapy, combining
chemotherapy with gene therapy, combining chemotherapy with immunotherapy and combining gene therapy with immunotherapy. Two or more therapeutic agents could be delivered through a single nanocarrier. This design is like a “double-edged sword”. Its advantage is that it can reduce the frequency of administrations and improve the comfort of the subject as well as avoid the influence of the interaction between carriers on the stability of agents. However, it is not possible to control the therapeutic dose combination of different therapeutics; it is impossible to select and optimize the timing of the different treatments, so it is difficult to maximize the combined therapeutic effect.

Based on the advantages of G23 functionalized curcumin-loaded zein nanoparticles shown in Chapter 2 to cross the blood-brain barrier and inhibit the growth of glioma cells, we evaluated the inhibition effect of combining zein-curcumin nanoparticles (CUR-NPs) and the chemotherapeutic temozolomide (TMZ) on U87 human glioma cells, and GG16 and GSC23 GBM neurospheres (Chapter 3). To increase the stability of TMZ in the physiological environment and prolong its circulatory half-life, TMZ was encapsulated in β-cyclodextrin by forming inclusion complexes (TMZ@CD). The combination treatment of CUR-NPs and TMZ or TMZ@CD showed a significantly enhanced inhibition of the proliferation and migration of U87 cells, and also markedly inhibited the proliferation and spheres forming capacity of GG16 and GSC23 neurospheres. We showed that induction of apoptosis contributes to the inhibitory effects of the combination treatment on GBM growth.

Based on the results from Chapters 2 and 3, we concluded that curcumin nanoparticles act as TMZ sensitizers and are promising for improving the treatment of GBM patients, which requires further in-depth mechanistic studies and validation in vivo glioblastoma models.

Gene therapy is a promising new strategy for treating various human diseases, including cancer, but the lack of a safe and effective gene delivery vector hinders its development. Viral vectors have problems such as immunogenic safety, limitation of target gene size and difficulty in large-scale production, while non-viral gene vectors have low transfection efficiency. A gene carrier must overcome several physiological barriers before it can deliver its cargo into tumor cells. First it has to be internalized by the tumor cell via endocytosis, and second it has to release the gene in the cell cytosol. Subsequently, the gene can be taken up by the nucleus, followed by transcription, and translation, resulting in expression of the gene. To obtain high transfection efficiency, the efficiency of the above steps are critical, which are influenced by many factors, such as gene carrier material, size, shape, and surface chemistry, and cell type. In Chapter 4, we studied the endosomal escape and gene transfection efficiency of a tertiary amine-functionalized $p$(NIPAM-co-DMAPMA) nanogel (NGs) and two quaternized nanogels (NGs-MI and NGs-BDD) in HEK293T cells. The quaternization of NGs-MI and NGs-BDD was obtained by alkylating the tertiary amino groups of nanogels (NGs) with methyl iodide and 1-bromododecane, respectively. Complexes of NGs, NGs-MI and NGs-BDD with pDNA at a weight ratio of 10/1 were formed, which exhibited high serum stability. Among them, only the NGs-BDD/pDNA complex showed endosomal escape and transfection efficiency in HEK293T cells. Because NGs-BDD showed high transfection efficiency, while its pH buffering capacity was negligible compared to that of branched PEI (25 kDa), i.e., a synthetic cationic polymer with high amine density, we further investigated its mechanism of action. Using bafilomycin A to prevent endosome acidification by blocking the V-ATPase pump, which has been proven to inhibit the transfection efficiency of PEI/pDNA complexes, i.e., PEI.
polyplexes\textsuperscript{15}, we found that the transfection efficiency of NGs-BDD complex was unaffected and therefore was independent of pH. We concluded that the long-chain alkyl groups in NGs-BDD help nanogels to escape from endosomes, which provides new ideas for enhancing the transfection efficiency of nanogels.

**Future Perspectives**

The experiments in this thesis showed that combined treatment of glioblastoma cells with curcumin-loaded zein nanoparticles and TMZ(\textsuperscript{@CD}) enhanced the efficacy compared to single treatment with either TMZ(\textsuperscript{@CD}) or CUR-NPs. Additional work is needed to understand the mechanism behind their combined action. In addition, \textit{in vivo} experiments are needed to investigate whether the two combined therapeutic agents have obvious pharmacokinetic interactions and whether the combined treatment of CUR-NPs and TMZ\textsuperscript{@CD} can achieve better GBM treatment effects than the single therapeutic agents. Likewise, the NGs-BDD nanogel may require optimization for \textit{in vivo} use. For instance, surface modification with e.g. polyethylene glycol (PEG) may be used to increase blood circulation time, although the low stiffness of nanogels may already promote long circulation time\textsuperscript{16}. In addition, the long-chain alkyl groups in NGs-BDD form hydrophobic domains that can be used to incorporate hydrophobic drugs. Therefore, it would be of interest to incorporate curcumin in the nanogel and combine it with gene therapy, and investigate the therapeutic effect on GBM.

To conclude, GBM is a complex and difficult-to-target disease, not only because of its secluded location within the brain, but also because of the complex tumor microenvironment (TME). We need to better understand the TME and use this knowledge to develop biomaterials that specifically kill GBM cells, leaving healthy tissue unharmed. Also we need \textit{in vitro} models that mimic the TME in order to investigate nanoparticle-mediated drug delivery for GBM treatment. Lastly, when designing drug and gene carriers, in addition to considering good therapeutic effects, we should also keep in mind the importance of low-cost, simple and large-scale production, to allow for the clinical translation of these carrier systems.
References


