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Review Article

Overview on Ligands for the Targeting in Treatment of Glioblastoma

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ABSTRACT

Glioblastoma is a grade IV malignant primary central nervous system tumor with poor prognosis. Radiotherapy chemotherapy, surgical resection are the current therapies use for the management of GBM, but it can also cause dangerous side effects hence targeted therapies are in development. In targeted therapies, receptor targeted drug delivery is one of the most successful strategy. A receptor which are more express over the cancerous tissue or blood brain barrier that can be easily targeted and it is advantage to differentiate cancerous tissue and normal tissue. We summaries receptor targeted therapies and reagent used for conjugating or coupling ligand to drug.

INTRODUCTION

Brain cancers in the central nervous system (CNS) are a heterogeneous group of primary and metastatic neoplasms, characterized by poor prognosis and low patient survival rate. These are categorized by the World Health Organization (WHO) according to a degree of malignancy closely related to diagnosis, ranging from grade I, characterized by lesions with low proliferative potential and cure potential, to grade IV, defined as cytologically malignant, mitotically active neoplasms typically associated with severe surround invasion. Glioblastoma (GBM) is a Glioma of type IV (Fig. 1) [1].

GBM is a grade IV malignant aggressive primary brain tumor with poor prognosis. The tumor originates from astrocytes and is extremely life-threatening in nature due to the rapid proliferation and penetration of tumor cells into different brain parts. The invasive nature of GBM makes it difficult to surgically remove a tumor [2]. The survival rate remains low for those diagnosed with GBM. Median overall survival is 15–23 months and 5 years is less than 6%, which is the lowest long-term survival rate of malignant brain tumors [3]. Current GBM management consist of surgical resection, followed by radiotherapy and adjuvant chemotherapy, both of which induce DNA damage [4].

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In addition, successful treatment of GBM allows anticancer agents to cross the blood-brain barrier (BBB) and accumulate therapeutically in tumor tissues; treatment without sufficient "drug uptake is likely to be ineffective [5]. In recent years, a number of strategies have been developed to overcome BBB and/or target glioma, such as receptor, transporter, or adsorption-mediated drug delivery according to different transport mechanisms [6, 7].

Receptor-mediated drug delivery has been extensively researched as one of the main approaches This type of drug delivery system includes of nanocarriers such as liposomes, nanoparticles (NPs), polymeric micells, dendrimers and polymersomes, and numerous ligands targeting specific receptors such as transferrin receptors (TfR), Lf receptors (LfR), LDLR, and folate receptors (FR). In this review we highlighted the importance of ligand in the management of GBM as a targeted drug delivery system. In particular we concentrated on the reaction involved in ligand and polymer or drug conjugation, and the impact of ligand conjugation over BBB or glioma cell and cancer cell cytotoxicity.

1. Ligand-Modified Targeting Systems:

The ligand-modified drug delivery systems are coupled with only one ligand which could target BBB and glioma, or only glioma cells. Wang Shanshan et al, 2015 Several receptors, such as transferrin receptor (TfR), Lf receptor (LfR),

folate receptor (FR), low density lipoprotein receptor (LDLR), insulin receptor (IR), have been reported to be strongly expressed in both BBB and glioma cells. Ligands are used to target receptor sites such as transferrin, Lf, folic acid, low density lipoprotein. The diagram below illustrates the ligand targeting receiver structure (Fig. 2).

Single Ligand- Targeting Systems

Transferrin

Transferrin As an important component of the human body, cellular iron plays a vital role in cell proliferation [8]. It consist of polypeptide chain of 679 amino acid, which is 78 KDa monomeric glycoprotein and member of glycoprotein family, it is of two types melano Tf and ovo Tf. The best characterized mechanism for iron uptake is mediated by the cell surface TfR, which is classified as TfR1 and TfR2 [9, 10]. Lots of studies have reported that TfR could be used for mediating drug delivery systems to the glioma.

Thiolation

Guo W et al 2013 examined the effect of resveratrol-conjugated transferrin-modified polyethylene glycol-polylactic acid (PEG-PLA) using traut's reagent for transferrin thiolation for glioma therapy.

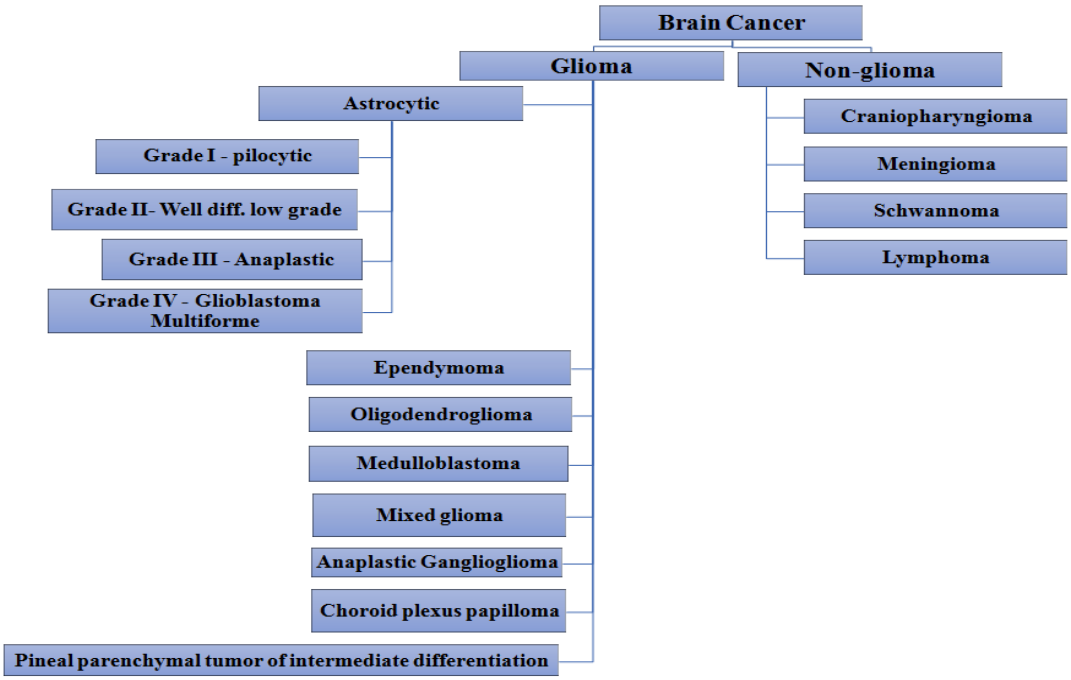

Figure 1: Classification of brain tumors

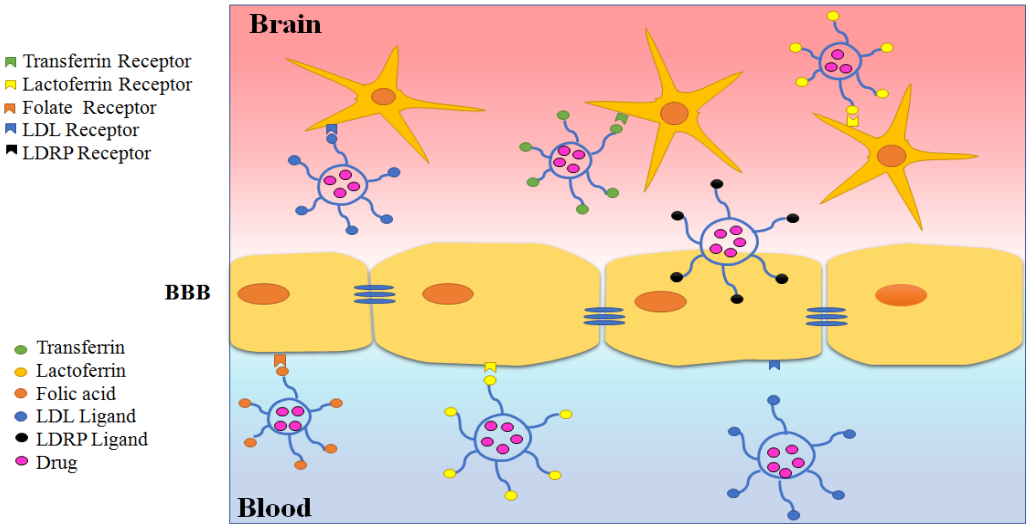

Figure 2: The ligand targeting receiver structure

In this study, In vivo biodistribution and antitumor activity in the Brain glioma bearing rat model of C6 glioma by intra peritoneal Administration of the conjugate RSV-polymer was reported. In vitro PEG-PLA-RSV cytotoxicity against C6 and U87 cells was reported to be higher than that of free RSV, and further Tf modification enhanced the cytotoxicity of RSV-polymer conjugates as a result of increased cellular uptake by glioma cells of the modified RSV conjugates. Compared with free RSV, RSV conjugates could significantly decrease the volume of tumors and accumulate in brain tumors, leading to prolonged survival of C6 glioma-bearing rats [11].

In another study the liposome of Transferrin / TAT was made as a model drug with doxorubicin. TAT conjugated doxorubicin-loaded liposome prepared and then combined with transferrin by using a trauts reagent for transferrin thiolation to treat glioma. Cellular uptake in both rat capillary endothelial brain cells (BCECs) and U87 cells was investigated. The Tf / TAT-lip-DOX is the best anti-proliferative action against cells in U87.The in vitro blood brain barrier model was established to assess the trans-endothelial ability that crosses the BBB. The orthotropic glioma model was established to evaluate the anti-glioma effect of in vitro experimental data, and in vivo indicated that the Tf / TAT-lip was a promising system for the delivery of brain drug delivery due to its high delivery efficiency across the BBB [12].

Hui-le GAO formulates polymersomes as a vector and was combined with transferrin using trauts reagent. In vitro, bEnd.3 cells used coumarin-6 as a fluorescent probe to investigate the uptake of Tf-polymersomes. The distribution of 125I-Tf-PS in vivo tissue and the pharmacokinetics were also examined. Tf-PS uptake by bEnd.3 cells has been shown to be time, temperature, and concentration-dependent. Tf increase polymerosomes s cell uptake to 37 °C [13].

Coupling

Afzal SM et al 2016, developed docetaxel lipid nanoemulsion coupled with transferrin to improve tumor targeted delivery. Transferrin was tagged using EDC reaction to stearylamine, which was containing lipid nanoemulsion globules. In vitro cytotoxic studies were performed on cells MCF-7 and HeLa. MDA-MB 231 cells were xenografted in Balb / c mice, and the efficiency of the tumor targeting was tested using imaging. In tumor targeting activity, transferrin coupled with docetaxel lipid nanoemulsion was found to be superior when compared to plain nanoemulsion [14].

Liu G et al 2013, developed transferrin coupled with PEGylated nanoscale graphine oxide for targeting glioma. Using EDC, transferrin was coupled with PEG-Graphine oxide. Glioma targeting delivery of the anticancer drug doxorubicin and Tf-PEG-GO-Dox was evaluated through systemic administration using C6 glioma cells and glioma-bearing rats. Studies both in vitro and in vivo showed that Tf-PEG-GO-Dox have a potential of nanoscaled drug delivery system for chemotherapy-targeting gliomas [15].

Dixit S et al 2015 developed TfR targeted theranostic gold nanoparticles for the delivery of photosensitizers in brain tumor. In this study PEGylated Gold nanoparticles have been conjugated via EDC / NHS to transferrin. In vitro studies of human glioma cancer lines (LN229 and U87) over-expressing the TfR showed a significant increase in targeted conjugate cell uptake compared to untargeted particles [16].

Jain A et al 2011 developed PEGylated Transferrin-appended nanoparticles for the delivery of temozolomide to brain. In this research, Transferrin coupled with PEGylated nanoparticles was prepared using sodium periodate (periodate method) by coupling transferrin to PEGylated nanoparticles. Research on the cytotoxicity of human cancer cell lines were performed in vitro. Confocal Laser Scanning Microscopy studies showed the enhanced uptake and location in brain tissues of Tf-appended PEGylated NPs [17].

Lactoferrin

Lactoferrin (LF) is an 80 kDa iron binding glycoprotein belongs to family of transferrin. Lf is a 703-amino acid glycoprotein that is a major component of milk and is present in neutrophil granules or other exocrine secretions, such as tears, saliva and cervical mucus [18, 19]. In adults, LF is synthesized by glandular epithelial cells and secreted into mucosal fluids that bathe the body's surface. Highest concentrations of LF are observed in colostrum and milk, with lower levels detected in tears, nasal fluid, saliva, pancreatic, gastrointestinal and reproductive tissue secretions [20].

The Lf receptors (LfRs) are vastly expressed on the surface of respiratory epithelial cells, brain endothelial cells, and neurons [21]. In addition, LfR has been shown to exist not only on the BBB

in different species, but also on the cell surface of glioma, which makes it a potential cascade-targeting ligand [22]. A number of studies have shown LfR-mediated enhancement of therapeutic effects against GBM. Lf is conjugated by various reaction such as thiolation, coupling.

Thiolation

Zhang J et al 2018, developed lactoferrin-and arginine-glycine-aspartic acid (RGD) dual-ligand-modified, temozolomide and vincristine-coloaded nanostructured lipid carriers for GBM combination therapy have been introduced. Lactoferrin-PEG-DSPE was synthesized by conjugating Lactoferrin to DSPE-PEG2000-MAL distal MAL functional groups. Lactoferrin was first thiolated with a Trauts reagent equivalent. L / R-T / V-NLCs displayed sustained release behaviour, strong cell uptake moderate cytotoxicity and synergy effects increased drug concentration in the tumor tissue, and apparent tumor suppression effectiveness with low systemic toxicity [23].

In another study, Hu K et al 2009 developed lactoferrin conjugated polyethylene glycol-polylactide. Lactoferrin was thiolated by the trauts reagent and conjugated to the distal maleimide function of the PEGylated nanoparticles to form lactoferrin nanoparticles. Significant increase in the uptake of lactoferrin nanoparticles by brain endothelial cells relative to nanoparticles by nanoparticles without lactoferrin [24].

Coupling

Kuo YC et al 2017 have developed catanionic solid lipid nanoparticles for the treatment of multiforme glioblastoma. In this research author loaded etoposide in solid lipid nanoparticles are modified with lactoferrin and wheat germ agglutinin by using EDC and NHS as coupling agent. The cytotoxicity study is performed on HBMECs and U87MG cells. Induced minor cytotoxicity to HBMECs, increased the ability of ETP to cross BBB by approximately 5.6 times, and improved the antiproliferative efficacy of U87MG cells.The modified WGA and Lf plays a crucial role in controlling ETP-CASLN's medicinal property, and WGA- Lf-ETP-CASLNs can be promising colloidal carriers in GBM management [25].

Chen H et al 2010, formulated procationic liposomes modified by Lf as the model drug carrier for the delivery of drugs targeted to the brain. In this study the author used Tris-EDTA buffer to modify procationic liposomes with lactoferrin. The primary capillary endothelial cells (BCECs) in the brain were developed to investigate the potential cytotoxicity and in vitro uptake of liposomes. The results showed improved efficiency and cytotoxicity compared to conventional liposomes and CLs, PCL and Lf-PCLs [26].

Folic Acid

Folate is a vitamin based on pterine, which has low molecular weight. Folate receptors (FR) are glycoproteins responsible for the binding of high affinity folate and subsequent transport to cells through endocytosis [27]. FR is highly expressed on the cell surface of cancer tissue compared to normal healthy tissue [28] . This suggests that folate is potential drug delivery system.

EDC Coupling

Feng S et al 2016 developed a targeted anticancer drug delivery based on folate conjugated boron nitrate nanosphere. In this study folic acid was grafted over the boron nitrate nanosphere by esterification reaction using EDC. In vitro cytotoxicity assay was performed on HeLa cells. BNNS-FA / DOX complexes exhibited higher cytotoxicity compared to free DOX and BNNS / DOX complexes, mainly due to increased DOX-mediated cell uptake [29].

Kuo YC et al 2019 formulated WGA and FA with etoposide carmustine and doxorubicin grafted with MPEG-PC NP to target BBB and inhibit human GBM development. In this study FA was grafted by EDC and NHS on MPEG-PCL NPs. WFNPs > FA-grafted NPs > WGA-grafted NPs > MPEG-PCL NPs followed an antiproliferative activity against U87MG cells for drugs [30].

Xu X et al 2016 established FA-BO-PAMAM as the validated tumor targeting carrier for anticancer drug DOX in this research. Using EDC as a condensation agent, FA conjugated to BO-PAMAM research shown that borneol alteration has effectively reduced PAMAM toxicity and improved BBB penetration so that FA-BO PAMAM / DOX had a beneficial impact on in vitro and in vivo glioma cells [31].

Pan J et al 2008 developed two component copolymer targeted delivery as a model drug, using paclitaxal. In this study EDC and NHS conjugated folic acid over TPGS-COOH. Research on cytotoxicity of C6 glioma cells conducted in vitro. Result showed that the formulation of nanoparticles vs the pristine drug has great

advantages, and folate decoration can significantly promote the drug's targeted delivery to the corresponding cancer cells and thus enhanced its therapeutic effects and reduced its side effects [32].

DCC Coupling

Niu J et al 2014 developed micelles to target brain tumor. In this study author conjugated folic acid to Pluronic P105 by using DCC and NHS. In vivo antitumor study was performed on mice-bearing C6 glioma model. in vivo studies showed that GF-DOX exhibited increased therapeutic efficacy and good safety with minimized weight change and cardiac toxicity [33].

Minaei SE et al 2019 formulated Folic Acid functionalized Temozolomide-loaded Poly (ethylene Glycol)–Poly (Butylene Adipate)–Poly (ethylene Glycol)-coated magnetite nanoparticles for targeted chemotherapy of glioma cells. Folic acid coupled with PEG-PBA-PEG by using DCC.Results from in vivo showed that TMZ-SPION-PEG-PBA-PEG-FA could be effectively slow down cell proliferation due to the targeting effect and high accumulation of TMZ in C6 cells via an FA-receptor mediated endocytosis [34].

Dual Ligand-Modified Targeting Systems

In dual ligand modified targeting system ligands play independent role to different targeting site. Dual ligand modified targeting system is for overcoming blood brain barriers and for targeting different receptors.

Gao JQ et.al 2013 developed dual targeting doxorubicin primed liposomes for targeting glioma and for penetration of blood brain barrier. Dual-targeting doxorubicin liposomes were formed by conjugating liposomes with both folate (F) and transferrin (Tf) by DCC as a coupling agent. liposome group were examined by using bEnd3 BBB models. In vivo experiments showed that the dual-targeting Dox liposomes could transport across the BBB and primarily spread in the brain glioma. These results show that this dual-targeting liposome can be used as a potential glioma chemotherapy carrier [35].

Kuo YC et al 2015 developed nanoparticles for transportation of etoposide across the blood–brain barrier (BBB) and glioblastoma treatments. EDC and NHS grafted the lactoferrin (Lf) and folic acid (FA) on (lactide-co-glycolide) (PLGA) nanoparticles. The antiproliferative efficacy of U87MG cells **against** growth was in the following order: Lf / FA / PLGA NPs > FA / PLGA NPs > PLGA NPs > Free etoposide solution [36].

For brain targeting Pang ZQ et al 2010 created lactoferrin- and transferrin-conjugated polymersomes. Lf and Tf were conjugated by the use of 2-iminothiolane over polymersomes. Using coumarin-6 as a fluorescent probe bEnd.3 cells were studied for in vitro uptake of Lf-PS and Tf-PS. Using a PS as the delivery vector and bEnd.3 cells as the blood-brain barrier (BBB) model, Tf was more effective in brain targeting than Lf [37].

CONCLUSION

For Glioblastoma, diagnosis and treatment various strategies are developed, amoung them receptor targeted drug delivery is one of the successful strategy. It reduces the dangerous side effect. The receptor targeting strategies are beneficial for differentiating cancerous cells and normal cells. The ligands are conjugated or coupled to drug molecule via conjugating or coupling agent such as trauts reagent, EDC, NHS, and DCC. From this review it is conclude that by conjugating or coupling ligand to drug molecule it reduces tumor by targeting receptor which are over expressed on cancerous cell.

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