Research Article

A Brief Review on Targeted Receptors for Immunotoxins in Immune Therapy For Glioma

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ABSTRACT

Immunotherapy is a new way for treating dreadful diseases like cancer. Now-a-days, activation immunotherapy is practiced in the case of cancer treatment. Activation immunotherapy is a type of immunotherapy where the immune system is stimulated to reject and destroy cancer cells. This review mainly focuses on immunotoxins as a method of immunotherapy in treating cancer. Immunotoxins are artificially constructed by inserting a specific toxin along with an artificial antibody. Each type of cancer will have its own receptors (antigens) on its surface and antibody to that antigen is constructed artificially and a specific toxin is linked with the antibody to kill the cancer cells. This method of using immune toxins in cancer treatment is a recent approach and is still under research. The efficiency of immune toxins in killing the cancer cell lines is high but a major drawback in using these immunotoxins is that they cannot penetrate into the cancer cell mass. So special delivery mechanisms would be required in delivering these immune toxins. The method of using immunotoxins is advantageous over other techniques as they only affect the cancer cells but not the normal cells. This review mainly focuses on treatment of glioma using immunotoxins and case studies on the treatment of Glioma and the results. Also it focuses on the design, construction, delivery and efficiency of various immunotoxins in treating glioma cells.

Keywords: Immunotoxins, Glioma, Monoclonal antibodies, Brain tumor, Tumor receptors.

INTRODUCTION

About 81% of the malignant brain tumors are Gliomas, the common intracranial tumors, with a relatively less survival rate. Mendelian disorders, including neurofibromatosis, tuberous sclerosis, and Li-Fraumeni syndrome are some of the causes for Gliomas.

With poor prognosis, GBM (Glioblastoma Multiform), a form of glioma usually recurs within 12 months after resection.[1,2]

In brief Immunotoxins are recombinant fusion protein conjugates coupled with cytotoxic agents targeting tumor-specific antigens.[3]

Immunotoxins are constructed by coupling cancer-cell Specific binding antibodies to a eukaryotic toxin.[4] Usually eukaryotic toxins such as anthrax toxin, ribosome inactivating toxin such as saporin and ricin, ADP ribosylating toxins such as diphtheria toxin, pseudomonas exotoxin (PE) are coupled to antibodies.[2,5] Usually these antibodies are monoclonal antibodies.[6,7] Such antibodies attached to protein toxins are called as recombinant immunotoxins that are usually used for cancer treatment.[8]

These recombinant immunotoxins are highly cytotoxic and are also apoptosis inducing and cell proliferation is affected.[2,5,8] In some cases the immunotoxin also inhibits the protein synthesis of the tumor cells.[9]

The targeted toxins are highly potent that even a single molecule is capable to cause cell death.[10] Immunotoxins can also act upon cancer cells treated with chemotherapeutic drugs like decarbazine.[11]

Immunotoxins which are difficult to produce, have also been produced in chloroplast of *Chlamydomonasreinhardtii.*[5]

Immunotoxins are tumor specific as they bind to cancer cells where antigens are over expressed.[11]

Angiogenesis, a requirement for tumor growth and metastasis is also targeted for treatment with conjugated immunotoxins.[12]

In case of Glioma, antigens such as GPNMB (Transmembrane Glycoprotein NMB), Membrane protein B7H3, Interleukin-4 receptors, RPTP beta (Receptor Protein Tyrosine Phosphatase), transferrin receptors are over expressed in cancer cells.[13-17]

In some cells, EGFR (Epidermal Growth Factor ReceptoR) gene and its mutant EGFR VIII gene are amplified and over expressed.[18]

Even Tumor associated Macrophages expressing FR beta (Folate Receptor) receptors are also targeted for immunotherapy which showed a decrease in tumor growth, but their promotion to tumor growth is unclear.[19] Parameters such as oxygen are also required for increased efficiency of the toxins.[20] An obstacle usually encountered with targeted toxins is the blood – brain barrier.[10] These targeted immunotoxins have significantly shown *in vitro* activity on many brain tumors.[21] Construction, delivery, mode of action, efficiency of immunotoxins for glioma treatment are discussed.

Mode of Action

The mode of action of various immunotoxins on various receptors are discussed here:

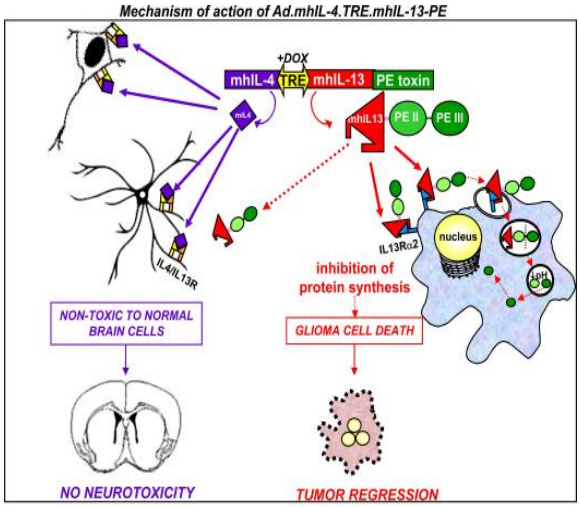

Figure 1: depicts that the Immunotoxin is harmless to normal cells and how it effectively kills the tumor cells.[9]

GPNMB Receptor

For glioma cells which highly express GPNMB, a mutant scFv (Single chain variable fragment) clone 902V which was derived from GPNMB specific G49 had a 11 fold increase in affinity for GPNMB. A mutant of this 902V clone was randomized through error prone PCR, F6V was selected by yeast surface display in which the light chain CDR2 exhibited the affinity. This was then fused with Pseudomonas exotoxin A to form F6V-PE38, which exhibited protein synthesis inhibition on GPNMB expressing glioma. Cytotoxicity on cells not containing GPNMB were negative.[13]

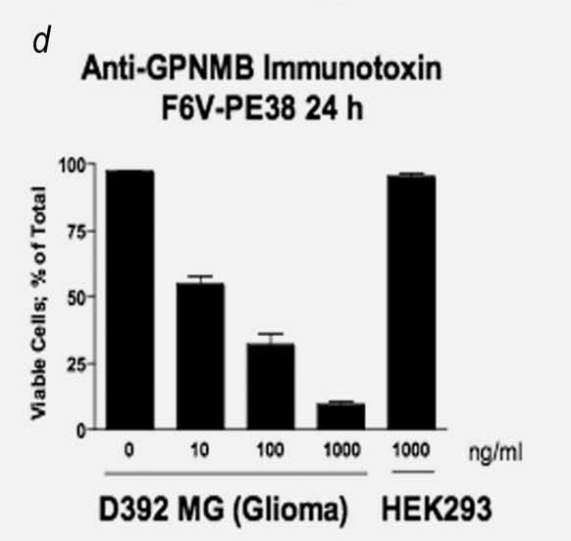

Figure 2: depicts Cell viability assay of various concentration of the immunotoxin on Glioma cell compared with a control HEK293 cells.[13]

EGF Receptor

EGF receptor gene [EGFR] along with its mutant EGFR VIII is overexpressed and amplified in glioblastoma cells. D2C7-scdsFv-PE38 kDEL immunotoxins reacts with 55 – amino acid region of the EGFR and EGFR VIII extracellular domain. The specificity of the immunotoxins was confirmed by flow cytometry for EGFR and EGFR VIII respectively. Specificity of the immunotoxins D2C7 was confirmed by flow cytometry with NR6W and NR6W cells. It was observed than convection-enhanced delivery of Immunotoxin in intracranial tumor models, such as 43, has prolonged the survival by 310%, thus proving a great potential for treating brain Tumors.[18]

U 87 Cell Receptor

In order to establish the immunotoxins IT 87, HFR2 and Two CDRS (Complementarity Determining Regions) ie LCDR2 and HCDR3 were fused to first 388 amino acids of Diphtheria toxin. It was observed that the antigen recognition of the parent antibody of the mimetic of LCDR, HF2, HCDR2 were retained. In *in-vitro* conditions, IT (Immunotoxin) 87 could kill U 87 glioblastoma cell line, the targets of the parent antibody but not the Raji cells. In SCID mice bearing both cells, the growth of the cells were inhibited in a treatment period of 20 days *in-vivo* as IT 87 targeted U 87 induced tumors.[22]

IL – 4 Receptor:

A wide variety of cancer cells expresses IL4 receptors, A chimeric Immunotoxin cpIL-4(13D)-PE38KDEL was constructed by fusing mute in cpIL-4(13D) to a modified pseudomonas exotoxin which showed improved toxicity on IL-4-R bearing cells. Tumor cells over expresses IL(Interleukin)-4 and IL(Interleukin)-13 on their surface. To target IL-4Rs and IL-13Rs, two recombinant fusion cytotoxin either IL-4 or IL-13 and mutated Pseudomonas exotoxin were fused to construct IL4-PEor IL13-PE which exhibited high cytotoxicity in *in-vitro* as well as *in-vivo* glioma models .But normal cells were not affected by the toxin.[23,24]

Immunotoxin IL4 (38-37)-PE38KDEL is composed of circularly permuted IL – 4 and truncated form of Pseudomonas exotoxin (PE). This was done by directly infusing the toxin in the glioma. After the administration of the toxin patients showed glioma necrosis, while Some patients had to undergo craniotomy due to increased cranial pressure. Some had partial to extensive tumor necrosis with edema patients who were not operated showed complete remission with extensive tumor necrosis. The patient was disease free for more than 18 months after the procedure. Direct administration of Immunotoxin is safe with no systemic toxicity.[26]

In a study, patients with recurrent malignant glioma were treated with IL-4 Pseudomonas Exotoxin (NBI-3001). The immunotoxin was administered intratumoraly through stereotactically placed catheters. No hematological or serum chemical changes of drug-related systemic toxicity were observed. Treatment related effects were restricted to the CNS. Even no death due to the toxin was seen. Areas of decreased signal intensity within the tumor consistent with tumor necrosis were observed in Gadolinium-enhanced magnetic resonance imaging of the brain in patients. So NB1-3001 is safe for administering intratumoraly.[30]

RPTP beta Antigen

Mechanisms such as cell proliferation, differentiation, communication, and adhesion are regulated by Protein tyrosine phosphorylation.

For several solid tumors Receptortype Protein Tyrosine Phosphatase beta (RPTPbeta) is a functional biomarker, whose expression is restricted to the central nervous system and astrocytic tumors, but a low expression in normal cells.

Monoclonal antibodies generated from mice, which recognizes RPTPbeta with low nanomolar affinities when fused with cytotoxinsaporin, killed glioma cells directly or via secondary antibody, which delayed Human U87 glioma tumors in mice xenograft models.[16,37]

Transferrin Receptor

Transferrin receptors and their interaction is important for the internalization and delivery of iron within the cell. A conjugate of human transferrin and a point mutated diphtheria toxin were coupled to form the Immunotoxin Tf-CRM 107. Also Tf (Transferrin) receptor monoclonal antibody and r-ricin, a conjugate 454A12-rRa was taken for study to determine their efficacy. In *in-vitro* both immunotoxins were potent and were specific for killing human glioblastoma cell lines. Nude mice model with U251MG flank was treated intratumoral with the above toxins in conjugated as well as non-conjugated forms. By day 30, tumors did not recur in cells where Tf CRM 107 was administered, and also the tumor volume decreased by 30 % with 454A12-rRa administration.

Tumor growth inhibition was seen with the non-conjugated forms but were less potent. The *in vivo* efficiency of targeted toxins against the glioma cells were seen from this study. With intratumoral administration these toxins were tumor specific and curative in some cases.[25,39]

Antigen Podoplanin

Podoplanin is a marker for cancer diagnosis mainly for head and neck carcinoma where its expression is upregulated. It is a transmembrane glycoprotein of 162 amino acids. In glioblastoma (D2159MG, D08-03308MG, D08-0493 MG) and Medulloblastoma (D283MED, D425MED, DAOY) xenograft and cell lines, the glioma tumor antigen podoplanin is present at very high levels. From the NZ-1 hybridoma, NZ – antibody specific for podoplanin was constructed, which was then fused with Pseudomonas Exotoxin to form NZ-1-(scdsFv)-PE38KDEL, where it is stabilized by disulfide bond between the variable regions. The Immunotoxin retained 38-98 % of its activity in a protein stability assay. *In-vitro* cytotoxicity of the Immunotoxin in glioblastoma and Medulloblastoma cell lines and xenograft were measured, which proved it to be highly cytotoxic with 1.6 – 29 ng/ml as the inhibitory concentration. 41 % increase in survival was seen in intracranial tumor model D425MED by the Immunotoxin.[27,36]

HWM –MAA Antigen

HMW-MAA (High molecular weight – Melanoma associated Antigen) is a membrane proteoglycan consisting of two sub units, was targeted for Immunotoxin treatment of Human glioblastoma Multiforme. To the antibody 9.2.27 which recognizes the HMW-MAA, the Pseudomonas exotoxin A was conjugated to form 9.2.27-PE. With a median inhibitory concentration of 1ng/ml, the Immunotoxin was cytotoxicto the U81MG cell line where the antigen was present. For intracranial U87MG tumors, administration of the 9.2.27-PE intratumoraly prolonged the survival in immuodeficient rats by 43%.[28,38]

Hypoxia

In a study it was found that anoxia or hypoxia altered the responsiveness of the cells to the Immunotoxin. Glioblastoma Multiforme cells, such as U-251 MG, U-373 MG, SNB-19, and A-172 MG were reoxygenated while treating them with cytotoxin, DT-IL13QM (Mutated IL13 based cytotoxin). Cytotoxic susceptibility increased when the cells were brought to normoxia, when compared to the cells maintained in normoxia. This brings an unexpected advantage by making the cells even more responsive to the cancer cells.[20]

B7H3 Receptor

B7H3 is a transmembrane protein present in gliomas and other cancer cells, where it has immunoglobulin like structure. It has both inhibitory as well as stimulatory effects on T cell activation. 8H9 antibody is specific for it and it is also reactive with most of the high grade human gliomas. The mean survival of rats bearing the xenograft U 87 was increased. By MRI, it was observed that the tumors showed volumetric response to the immunotoxin. Interstitial infusion of the immunotoxin serves to be potential in treating the Glioma of the brain stem and the hemisphere (Refer Figure 3).[14,35]

Angiogenesis

Inhibiting the tumor neovascularization also had effects in solid tumor growth. In a study it was observed that the immuotoxin VEGF165-PE38 had a direct inhibition of angiogenesis in chorioallantoic membrane in chick and it was applied for malignant glioma models. The plasmid containing the immunotoxin ie.pVEGF165PE38-IRES2-EGFP was directly administered in murine malignant glioma models via multiple local intratumoral delivery. The tumor volume in mice treated with the plasmid for the immuotoxin was significantly lower than the control groups by day 16 . Analysis of histo chemistry has shown that the decreased expression of CD31 in treated animals.[29]

EphA2 receptor

EphA2 is a receptor which is mostly expressed in *in vitro* and *in vivo* epithelial cells and is over expressed in human cancer cells, where in glioma cell lines chemotactic cell

migration and invasion is increased when EphA2 is over expressed. One of the major problems that was encountered with immunotoxins was that they cannot penetrate the tumor mass but they were effective with tumor cell lines. Tropism was exhibited by Mesenchymal Stem Cells to the tumor tissue so they can serve as cellular vehicle for the delivery of antitumor agents. EphA2 receptor is over expressed in Gliomas which is targeted by the Immunotoxin Epinephrin A1 – PE 38. Transduced with Adeno virus the Mesenchymal Stem Cells were made to express secretable Epinephrin A1 Immunotoxin. Production of immunotoxins by hMSCs were confirmed by *in-vitro* assays .Immunotoxins produced had selective killing effect which inhibited the tumor growth in Glioma models.[31,34]

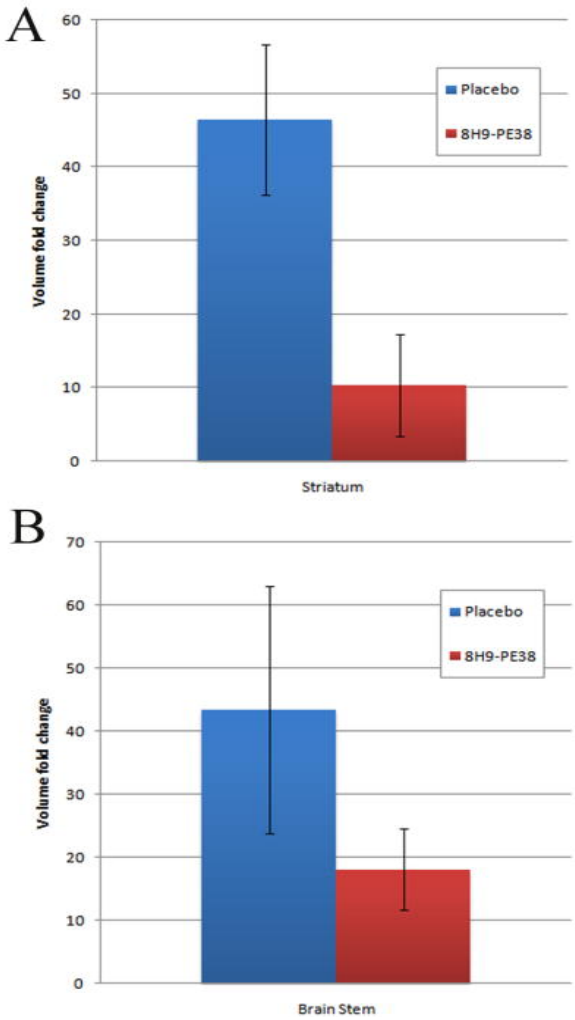

Figure 3: U87 Xenograft volume-fold change (with standard error) as determined by MRI performed two weeks following treatment with 8H9-PE38 versus placebo in the striatum (A) and brain stem (B).[14]

CONCLUSION

Immunotoxins are very effective in treating cancer cells as they do not involve normal cells. So it is advantageous over other treatments such as chemotherapy, radiation therapy etc. Even the efficacy of immunotoxins in treating cancer cells are high as 90 % for tumor cells lines and between 70 % and 90 % in the case of tumor mass. Soon as clinical trials are over, this form of treating with toxins find great potential in treating patients with various forms of Glioma. Not only that, Immunotoxins can also treat various kinds of cancer too. So this treatment has a great potential in the near future with less complication.

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