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Molecular Targets for Therapy in Malignant Gliomas

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I MALIGNANT GLIOMAS: WHAT ARE THEY?

Glial cells are the supporting cells of the central nervous system (CNS) and are the most abundant cell type in CNS. Their role is to nurture and sustain the neurons. They are the mediators of cross-talk between neurons and endothelial cells, and respond to stimuli through signaling pathways. Glial cells are of three sub types, astrocytes, oligodendrocytes and microglia. Glial tumors is a broad term encompassing tumors arising from astrocytes (astrocytomas), oligodendrocytes (oligodendrogliomas), and ependymal cells (ependymomas) (Srivastava et al 2007). Glial tumors or gliomas are a group of highly heterogenous tumors and also the most common neoplasms of the brain (Maher et al 2001). They are the second most frequent cause of neoplasm in young adults after hematological malignancies. The major group of malignant glioma in the CNS is the Grade III Anaplastic Astrocytoma (AA) and the highly malignant form Grade IV Glioblastoma Multiforme (GBM). Most GBMs appear de novo, although approximately 10% have a prior clinical history of a lower grade astrocytoma, in which case they are also termed secondary GBMs

II CURRENT THERAPEUTIC REGIME FOR GLIOMAS

The outcome of malignant gliomas has changed only marginally despite vast improvements in imaging and non-invasive early detection as well as in treatment modalities such as chemo/radiotherapy. The life expectancy of GBM patients after diagnosis has improved to the present average of 14 months. These highly heterogeneous groups of tumors are difficult to treat and recurrence is a constant threat. The treatment of GBM largely remains the same - surgery followed by radio/chemotherapy. While the type of radiation used has not changed, there have been vast improvements in the execution of the radiation and the beam can now be intensity-modulated and image-guided to greater precision than before (Van Meir et al 2010). The chemotherapeutic drug of choice is now Temozolomide which is more tolerable by patients (Villano et al 2009). A well researched treatment regimen exists for GBMs and it includes complete excision, followed by radiation with concomitant temozolomide and followed by monthly cycles of temozolomide (Stupps et al 2002). Variations in this regimen exist mostly in increasing the concomitant dose, or continuing the monthly cycles till recurrence. For AA, a similar protocol is followed. It is only very recently and after extensive research that targeted therapy has finally found its way into clinical practice, and bevacizumab, an antibody against vascular endothelial growth factor (VEGF) has been approved for the treatment of recurrent or progressive glioblastoma. The current basic and translational research objective in the field of neuro-oncology is in further discovering and developing therapeutic molecules against such specific targets.

III DRUGS IN CLINICAL PHASE TRIALS

A number of studies have contributed to testing various small molecules in improving the prognosis of this disease. Delineating pathways of proliferation, apoptosis and metastasis have provided researchers with molecular targets which could help in developing small molecule inhibitors and modulators. Identifying new markers and segregating large data into a pattern using various genomic approaches and clinical correlates has helped in defining a molecular characterization of GBM into subtypes. Frequent mutation in p53 and activation of Ras and tyrosine kinase pathways still remains a common feature in a large number of these tumors (Verhaak et al 2010). Identification of mutations in Isocitrate dehydrogenase (IDH1) is a relatively new discovery and has helped in this characterization (Zhao et al 2009). In addition, neural and precursor stem cell markers (Zheng et al 2008) of Notch and Sonic Hedgehog signaling pathways are a subject of great interest especially with the proposition of differences in cell type origin. Hypoxia inducible factor (HIF), Insulin like growth factor binding proteins 2 (IGFBP2), Platelet derived growth factor receptor (PDGFR) and Epidermal growth factor receptor (EGFR), stem cell markers such as Nestin, and stress chaperones (HSP90) are now being investigated as

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attractive targets for glioma therapy. Some of these small molecule inhibitors and targeted antibodies being tested in various phases of clinical trials against some targets are discussed below.

III A. KINASE INHIBITORS IN GLIOMA THERAPY

Against EGFR and its Mutant EGFRv111

EGFR (7p12) is a 170 kDa transmembrane tyrosine kinase that specifically binds the 53–amino acid peptide ligand, epidermal growth factor and the 50–amino acid autocrine growth factor, transforming growth factor-α (Mendelsohn et al 2006). The gene is known to be overexpressed in nearly 40% of the primary GBM. Mostly the tumors which show EGFR overexpression also show the presence of a mutant form of the receptor, EGFRvIII, which has an in-frame deletion of exons 2 to 7 of the extracellular domain of the *EGFR* gene (Pedersen et al, 2001), is expressed and amplified in up to 57% of GBMs and in 75% of anaplastic astrocytomas (Wikstrand et al 1998, Pelloski et al 2007). Presence of EGFRvIII gives it a specificity for tumor targeting and a number of inhibitors and antibodies have been developed for diagnostic and therapeutic purposes.

A number of tyrosine kinase inhibitors (TKI) have entered various phases of clinical trials. Two such inhibitors, Gefitinib and Erlonitib, showed promise in initial stages of trials however, the response was highly variable in these heterogeneous set of tumors. While some tumors responded well to the TKI, others were additionally dependent on the status of other molecules. In a glioma culture enriched for tumor initiating (cancer stem cells), both Gefitinib and Erlotinib were shown to have no effect on cells with mutant PTEN and activated Akt (Griffero et al 2009). After considerable research on HER (Herceptin) /EGFR inhibition, the favorable concept now is of a multiple target approach concomitantly aimed at different molecular sites. Other TKI being investigated are Lapanitib (Giannopoulou et al 2009), PF-00299804 and BIBW2992 (the last two being irreversible inhibitors currently in Phase I efficacy trials).

Against VEGF Signaling

Angiogenesis is a multistep process and in cancer, the development of new blood vessesls is an important factor for metastasis. VEGF is a major growth factor for endothelial cells. (Coultas et al., 2005). VEGF is a ligand for VEGFR1 and VEGFR2. VEGFR2 appears to be the main mediator of VEGF-induced endothelial proliferation, survival, migration, tubular morphogenesis and sprouting in adults (Ferrara et al., 2003). The VEGFR signaling, activated by the binding of VEGF and subsequent dimerization of receptors, recruit signal-transduction molecules such as PLC, PI3K and Ras (Olsson et al., 2006). The VEGF mimetics, inhibitors of downstream molecules and negative regulation of VEGFR2 signaling are important in limiting the response of VEGF in target cells.

CT-322, is one such specific VEGFR2 inhibitor which is in Phase II clinical trials alone and in combination with Irinotecan (chemotherapeutic drug). It binds to and inhibits VEGF2. CT-322 is an adnectin. These small proteins are derived from type III domain of human fibronectin, an extracellular protein that is abundant in human serum and the extracellular matrix, and naturally binds to other proteins. When the amino acid sequence of its targeting loops are modified for specific targets, the Adnectin shows nanomolar or picomolar affinity, and potency and specificity comparable to or better than antibodies (Getmanova et al 2006, Parker et al, 2005). Vandetanib is a dual inhibitor against VEGFR2 and EGFR and is showing tremendous promise in Phase II/III clinical trials (Morabito et al 2009). XL-184, Pazopanib, Sorafenib and Sunitinib are a few other TKI which are being investigated for high grade and recurrent glioma treatment.

Against PDGFR

Platelet-derived growth factor (PDGF) is known to promote cell migration, proliferation, and survival by binding to their tyrosine kinase receptors, PDGFRα and PDGFRβ. PDGFRs and/or their ligands has been described in many other solid tumors such as medulloblastomas and malignant gliomas (MacDonald et al 2001, Kho et al 2004, Jones and Cross, 2004). Therefore, PDGFRs are attractive targets for anticancer therapeutics.

Imatinib, a PDGFR inhibitor (Gleevec, STI-571), has benefited patients with myeloid malignancies and gastrointestinal stromal tumors (GIST). While the Imatinib is in Phase III clinical trials for GIST, it is still in Phase II trials for gliomas (Wen et al 2006). Dasatinib, another TKI, is in Phase I/II clinical trials for recurrent glioma. Tandutinib, a piperazinyl quinazoline receptor TKI, is currently in Phase II trials in combination with other drugs (Bevacizumab) for recurrent glioma.

Other targets

mTOR signaling pathway is implicated in response to stress and growth factors and is linked to the PI3K/AKT pathway. AKT is known to phosphorylate mTOR (Stokoe and Furnari, 2009). A number of mTOR inhibitors such as Sirolimus (rapamycin), Everolimus (RAD001), Temsirolimus (CCI-779) and Ridaforolimus (AP23573) are under study (Van Meir et al, 2010). Cilengitide

(EMD121974) is a synthetic cyclic pentapeptide mimicking the Arg-Gly-Asp (RGD) binding site with specificity for Integrins αvβ3 and αvβ5, which are highly expressed in glioma. Aflibercet is an interesting molecule for inhibiting the VEGF signaling. In this segments of the extracellular domains of human vascular endothelial growth factor receptors 1 (VEGFR1) and 2 (VEGFR2) were fused to the constant region (Fc) of human IgG1 with potential antiangiogenic activity. The molecule hence acts like a decoy receptor, binds to VEGFs and prevents their binding to cell receptors (Tew et al, 2010).

III B. MONOCLONAL ANTIBODIES IN GLIOMA THERAPY

Drug Delivery

Monoclonal antibodies provide a specificity and efficacy to targeted therapy which is much higher than those of inhibitors. Their mechanism of action is simple: binding to the target and rendering it inaccessible for ligand/receptor binding and hence preventing downstream signaling. However, monoclonal antibody (mAb) therapy has to overcome a number of barriers for it to be successful. Developing non-immunogenic mAbs (ie, mAbs that do not generate a human anti-mAb antibody or HAMA response) is one such major factor and requires considerable optimization. Another major difficulty is standardizing the route of administration and drug delivery (Clarke et al 2009). mAb therapy in glioma is often intratumoral rather than systemic. Catheter or convection-enhanced delivery methods are generally used for targeting and reducing toxicity to other tissues. Briefly, this involves optimization of the catheter used for delivery and the pressure applied such that more efficient diffusion of the drug is given to difficult to reach interstitial spaces within the tumor tissue (Saito et al 2004, Yang et al 2009)

Target and their mAbs

EGFR and its mutant EGFRvIII continue to be a major target for mAb therapy. Cetuximab, a chimeric mAb against EGFR, is in clinical phase I/II trials in combination with other molecules and chemo/radiotherapy. It has already entered Phase III trials in non small cell lung cancer. Researchers have also moved a step ahead by developing peptides (mimotopes) against Cetuximab such that they may be developed for an enhanced immune response against tumor cells when given in combination with Cetuximab (Hartmann et al, 2010). The group also conducted a similar study with Matuzumab, another anti-EGFR mAb which was previously abandoned due to poor Phase II study results. Nimotuzumab, a humanized mAb against anti-EGFR, has also been approved for glioma and has entered Phase III trials in Germany for pediatric gliomas. In India, Phase I/II trials are on-going for head and neck cancers (Ramakrishnan et al 2009). Nimotuzumab is expected to show less toxicity to normal tissue due to its inherent property of bivalent binding (i.e., binding with both antibody arms to two targets simultaneously) for stable attachment to cellular surface. As a consequence, in contrast to Cetuximab, which shows high avidity, Nimotuzumab selectively binds to cells that express moderate to high EGFR levels as observed in tumor cells.

Bevacizumab is the most successful mAb for glioma treatment and is a full length humanized IgG directed against VEGF. This was approved as a second-line treatment against glioma in 2009 much after its approval for metastatic colorectal cancer in 2004, an indication of the problems associated with accessibility and delivery in brain cancers.

Apart from the relatively successful anti-EGFR and anti-VEGF mAb, other targeted antibodies have also gained ground. Ramucirumab or Anti-PDGFRα mAb IMC-3G3 is currently in Phase II trials for recurrent gliomas. Phase I and II clinical trials of the [131]I-labeled murine anti-tenascin monoclonal antibody 81C6 have also been conducted in patients with newly diagnosed malignant glioma (Cokgor et al 2000, Reardon et al 2002 and Bigner et al 1998). Tenascin is an extracellular matrix glycoprotein that is expressed in malignant gliomas but not in normal brain tissue.

III C. IMMUNOTOXINS: mAbs WITH A LICENSE TO KILL

When targeted antibodies are linked with a toxin (bacterial/plant) then ingestion of the antibody by the receptor allows localized delivery of the toxin to the cell. Unique or over-expressed cell surface receptors on GBM cells are targeted with a receptor-specific immunotoxin for targeted therapy. Over the years, Puri's group at NIH has worked towards developing immunotoxin from an over-expressed receptor for an immune regulatory cytokine, interleukin-13 (IL-13). They observed overexpression of the receptor on human malignant glioma cell lines, primary brain tumor cell cultures, and tumor tissues. The targeting of IL-13 receptors (IL-13R) with a recombinant fusion protein composed of IL-13 and a mutated form of *Pseudomonas* exotoxin (IL 13-PE3 8QQR or IL-13-PE38, referred to here as IL 13-PE) demonstrated apotent and specific cell-killing of GBM cells in vitro. The immunotoxin was given using convection enhanced delivery system. After a successful PhaseI/II trial, the IL-13-PE has entered Phase III clinical trials (Kioi et al 2006, Joshi et al 2000, Kawakami et al 2002).

The Transforming growth factor alpha PE38 (TGFα-PE38), composed of TGFα and a mutated form of the *Pseudomonas* exotoxin termed PE38, has been shown to be toxic to cells in proportion to EGFR expression (Phillips et al 1994). A recently completed phase I trial of TGFα-PE38 delivered by convection-enhanced delivery to patients with recurrent malignant brain tumors has demonstrated no systemic toxicities, with an overall median survival of 23 weeks (Sampson et al 2003)

PRX321 (Protox Therapeutics Inc), Il-4-PE immunotoxin targets the highly expressed IL-4 receptors on GBM and recurrent GBM. This immunotoxin is currently in the middle of its Phase II trials for high grade and recurrent gliomas. Similarly Cotara™ is a mAb which targets dying cells (exact details not revealed by the company) and is currently in Phase II clinical trials.

IV CONCLUDING REMARKS

With better molecular characterization and understanding of the various pathways of glial tumorigenesis and progression, new biomarkers are emerging. These can be developed into potentially lethal specific targets to glial tumor cells. While the biggest drawback in glioma therapy remains that of drug delivery and reduced efficacy of drugs, a combination of assisted therapy (invoking the immune response) and better targeting strategies, hold tremendous promise in the future for improving the quality of life and survival in glioma patients.

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