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REVIEW

Targeting integrins in malignant glioma

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Abstract The integrin family of cell adhesion receptors is emerging as a promising target of anticancer therapy. AlphaVbeta3 and alphaVbeta5 integrins are overexpressed on both glioma cells and tumor vasculature. Cilengitide, the most advanced specific integrin inhibitor in oncology, has shown antitumor activity against glioma in early clinical trials. Durable remissions have been observed in phase I and phase II trials for recurrent glioblastoma (GBM) with both lower and higher doses of cilengitide. Pilot trials in newly diagnosed glioblastoma in conjunction with standard chemoradiotherapy have been encouraging. Preclinical data suggest synergy with concomitant chemo- and radiation therapy. A pivotal phase III study (CENTRIC) in newly diagnosed GBM patients is currently recruiting. This paper

summarizes the current understanding of the role of integrins and their inhibition in gliomagenesis. The background and design of ongoing trials are outlined.

Keywords Integrins · Angiogenesis · Glioma · Glioblastoma · Clinical trials · Review

Introduction

Integrins are heterodimeric transmembrane cell surface receptors that play a key role in the crosstalk between the cell and its surrounding stroma [1]. Twenty-four different integrins have been identified to date. Integrins link the

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cytoskeleton to the extracellular matrix, and are recognized to be key regulators of tissue structure. Integrins regulate cell adhesion, migration, differentiation, proliferation, and survival during physiological and pathological conditions, including inflammation and cancer. Upon ligation to extracellular ligands (i.e., matrix proteins such as collagens, laminins, vitronectins and fibronectins), integrins activate downstream signaling pathways in concert with growth factor receptors, including platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR).

Preclinical data indicate that integrins play a key role in cancer initiation and progression [2]. They provide adhesive, migratory, and survival cues to tumor cells and to cells of the tumor microenvironment, including angiogenic endothelial cells. The integrins α V β 3 and α V β 5, among others, are highly expressed not only on the tumor vasculature and angiogenic endothelial cells, but also on tumor cells, including gliomas (reviewed in [3, 4]). Consequently, integrins have been considered as a promising therapeutic target in cancer [5]. Monoclonal antibodies and peptide-based integrin inhibitors are being investigated for their potential therapeutic activity in various tumor types. This strategy is in advanced stage clinical development in glioblastoma, a highly vascular primary brain tumor. The only integrin inhibitor being studied in glioma is cilengitide, we therefore focus this short review on this agent.

Preclinical data on integrins in glioma

In high-grade glioma, and in particular in glioblastoma, overexpression of α V β 3 integrin is well documented. Importantly, α V β 3 integrin is expressed both on angiogenic endothelial cells and on tumor cells [6–8]. Molecular imaging of α V β 3 expression using the tracer [18F]Galacto-RGD and validation by immunohistochemistry revealed that α V β 3 integrin expression was mainly confined to the tumor region and was absent in normal tissue [9]. Selective α V β 3 upregulation in malignant gliomas suggests that integrin signaling in glioblastoma has important functions. Emerging evidence indicates that integrins promote glioblastoma adhesion, migration and angiogenesis [10, 11]. In an autocrine loop hypoxia will recruit α V β 3 and α V β 5 integrins and activate focal adhesion kinase (FAK). Integrin inhibition decreases hypoxia-inducible factor 1 α (HIF-1 α) and reduces thus tumor hypoxia, which may lead to increased radiation sensitivity [12].

The integrin α 6 β 1 plays an important role for the regulation of glioma-initiating cells in the perivascular

niche [13]. This integrin mediates the interaction of glioma-initiating cells to laminin, an extracellular matrix protein expressed in basement membranes, including those supporting endothelial cells. This interaction provides an anchorage for glioma-initiating cells within the perivascular niche and supports their tumorigenic potential.

Given the role of integrins in promoting glioma growth, invasion and angiogenesis, integrin inhibitors might be ideal therapeutic tools with synergistic activities in conjunction with already established therapeutic modalities, i.e., radiation therapy and cytotoxic chemotherapy. Importantly, because of their antiangiogenic effects [14], integrin inhibitors are expected to normalize the tumor vasculature, which in turn might enhance the effects of radiation therapy and chemotherapy. Indeed the efficacy of these two therapeutic modalities depends on an effective blood perfusion for oxygenation of the tumor (i.e., an important condition for radiation-induced tumor cell killing) and optimal drug delivery. Inhibition of α V β 3 and α V β 5 integrins in hypoxic glioma cells by siRNA decreases HIF-1 α by regulating FAK. Silencing this signaling cascade in established xenografts indeed reduced hypoxia and decreased angiogenesis [12].

Interestingly, ionizing radiation induces ceramide-mediated apoptosis of tumor endothelial cells, causing tumor vessel disruption and delayed tumor growth [15]. Consistent with a cytotoxic effect of radiotherapy on angiogenic vessels, concomitant administration of antiangiogenic drugs decreases endothelial cell survival. Anti-VEGF antibodies (e.g., bevacizumab) [16], VEGFR inhibitors (e.g., AG-013736) [17], mTOR inhibitors (e.g., RAD001) [18], and integrin inhibition (cilengitide) [19] sensitize angiogenic endothelial cells to ionizing radiation-induced death, thereby enhancing tumor vascular damage induced by radiotherapy and improving therapeutic response [20]. Irradiation of glioma cells increases their expression of α V β 3 integrin [21], thus integrin inhibition will further synergize with radiation therapy.

Regarding clinical translation, targeting α V β 3 and α V β 5 might reduce hypoxia and thus resistance to radio- and/or chemotherapy [12]. Indeed, arresting integrin signaling synergistically enhances the anti-glioma effect of radiation therapy *in vivo* [22].

A recent preclinical study suggests that low concentrations of small molecular integrin inhibitors used as single agents may stimulate angiogenesis and tumor growth in experimental melanoma and Lewis Lung carcinoma models, through increased VEGF expression and VEGFR2 signaling [23]. The concentrations of the small molecular integrin inhibitor cilengitide, measured in patients in clinical trials, however, largely exceed the critical “proangiogenic” concentrations described in this preclinical study, and therefore, paradoxical proangiogenic effects of small

molecular integrin inhibitors in the clinical application seem rather unlikely [24]. In fact, relative cerebral blood volume and blood flow, measured by perfusion MRI, remained stable or decreased among recurrent GBM patients responding to cilengitide therapy in a prior phase I study [25]. Durable and clinically relevant responses have been observed with cilengitide monotherapy; ongoing clinical studies administer cilengitide in combination with radiotherapy and/or chemotherapy. Nevertheless, the durable therapeutic success will depend on the efficient management of escape mechanisms occurring under anti-integrin therapies [26, 27].

Integrin inhibition attenuates invasion and metastasis secondary to radiation-induced angiogenesis inhibition

Vascular normalization may not be the only mechanism by which integrin inhibitors synergize with radio-chemotherapy. We have recently observed that high doses of ionizing radiation locally suppress angiogenesis *in vivo* and inhibited endothelial cell sprouting *in vitro* through a cell-autonomous effect mediated by the TGFbeta receptor ALK5 [28]. Tumors derived from oral squamous cell carcinoma and from hepatoma cell lines growing within a pre-irradiated bed had decreased microvascular density and were smaller, but their margins were highly invasive. They also had increased hypoxia and necrosis, and a more aggressive behavior with a higher number of lung metastases [29]. Thus, pre-irradiation of the tumor bed reduces local tumor growth, likely due to acute metabolic starvation secondary to suppressed angiogenesis, but at the same time it may enhance local invasion and metastatic spreading, possibly through increased hypoxia. We have identified the cysteine-rich protein 61 (CYR61), a member of the CCN (CYR61/CTGF/NOV) family of matricellular proteins regulating cell growth, differentiation, survival and migration in development, tissue remodeling and repair [30], and integrin alphaVbeta5 (a receptor for CYR61) expressed by tumor cells, as critical molecules that cooperate to promote local invasion and distant metastases [29]. Importantly, function-blocking anti- α V mAb 17E6 [29, 31] and cilengitide-inhibited CYR61-mediated invasion and metastasis formation of tumor cells growing in a pre-irradiated bed. These results suggest that cilengitide may improve cancer control in conjunction with radiotherapy by attenuating some of the unwanted rebound effect of radiation-induced inhibition of angiogenesis. Hypoxia is well known to activate pro-invasive programs [32] and select for resistant variants with invasive characteristics. This view is consistent with a recent experimental study demonstrating that selected inhibition of tumor angiogenesis by antiangiogenic drugs, including in experimental

glioma, results in increased invasion and metastasis formation [33].

Cilengitide

Cilengitide (Merck KGaA, Darmstadt, Germany) is a synthetic Arg-Gly-Asp (RGD) pentapeptide recognizing the RGD ligand-binding motif (ligand binding site) on the integrin receptors alphaVbeta3 and alphaVbeta5 [34, 35] and competitively blocks integrin ligand binding. It was shown to diminish angiogenesis *in vitro* [36]. In an important early preclinical study, cilengitide markedly suppressed tumor growth in a medulloblastoma and orthotopic glioblastoma models (i.e., tumors were grown in the brain), while no growth inhibition was demonstrated in a heterotopic model (i.e., when tumors were grown in the flank of nude mice), or when an inactive peptide was used [37]. This suggests that the brain environment is particularly susceptible to integrin inhibition and led to subsequent clinical investigation.

In phase I studies cilengitide was administered twice weekly by intravenous infusion over 1 h. No dose limiting toxicity was observed with doses up to 2400 mg/m². Peak plasma concentrations that had shown antitumor effects in preclinical models were achieved at doses ≥ 120 mg/m² [25, 38–40]. The terminal half-life of cilengitide is 3–5 h, suggesting the possibility of increased efficacy with a more frequent administration or continuous infusion schedule.

Clinical experience with cilengitide in malignant glioma

Sustained responses in recurrent glioblastoma were seen both with lower and higher doses of cilengitide as single agent in two phase I trials in adult and pediatric patients [25, 39]. Overall cilengitide was well tolerated, a maximal tolerated dose not reached and a clinical benefit seen both at higher and lower doses. Subsequent trials were initiated with flat (not per m²) twice weekly dosing of cilengitide at an intermediate lower (500 mg) and intermediate higher (2000 mg) dose (Table 1). In a randomized phase II trial of 81 patients with recurrent glioblastoma, treatment was administered at the lower and higher dose. Cilengitide monotherapy was well tolerated independently of the dose. Objective responses were achieved in 5% or 13% of patients treated with 500 mg or 2000 mg, respectively. Progression-free survival after 6 months was 10% and 15%, whereas overall survival was in the range of 6.5 versus 9.9 months, respectively [41]. Recently, long-term survival results were reported demonstrating a 4-year survival rate of 2.4% (95%CI 0.2; 11%) in patients treated with the lower dose, and 10.0% (95%CI 3.2; 21.5%) in patients

Table 1 Completed trials of cilengitide in primary brain tumors

Author/year (trial#)	Trial design/number of patients	Cilengitide dose ^a	Disease setting	Main results
Nabors/2007 (NABTT9911) [25]	Phase I, 51 patients (38 with GBM)	Single agent 120–2400 mg/m ²	Recurrent malignant gliomas	No DLT and MTD; no bleeding events 2 CR (1 GBM, 1 AA) lasting 12 + 24+ months 3 PR (mean duration of 9.3 months), 16 SD (mean duration of 5.4 months)
MacDonald/2008 (PBTC012) [39]	Phase I, 31 patients	Single agent 120–2400 mg/m ²	Pediatric refractory gliomas, meningiomas, and PNET	No MTD; intratumoral hemorrhage (asymptomatic, grade 1 in 2, grade 3 in 1 patient at 2400 mg/m ² dose level). 1 CR (GBM, lasting >1 year) 6 SD (lasting >1 year in 3 pts)
Gilbert/2008 (NABTC 03-02) [44]	Translational phase II, 30 GBM patients	Single agent 3 doses (500 mg or 2000 mg) preop. until day -1. Postop. therapy at 2000 mg	Recurrent glioblastoma planned for second surgery	No bleeding complications, no wound healing complications in subsequent surgery Cilengitide detected in all tumor samples, trend to higher tumor exposure with higher dose. PFS-6 12%
Reardon/2008 (EMD009) [41]	Randomized phase II, 81 GBM patients	Single agent 500 mg versus 2000 mg	Recurrent GBM (first recurrence after TMZRT failure, measurable disease)	500 mg: RR 5%; PFS-6 10% (CI 3–24); median OS 6.5 (CI 5–9); 2000 mg: RR 13%, PFS-6 15% (CI 6–30), median OS 9.9 months (CI 6–16)
Nabors 2009 (NABTT0306) [43]	Randomized phase II 112 GBM patients	500 mg versus 2000 mg in combination with TMZRT→TMZ	Newly diagnosed GBM	median OS 18.9 months (CI 17–22), median OS for 500 mg cohort: ~17 months median OS for 2000 mg cohort: ~21 months
Stupp/2010 (EMD010) [45]	Phase I/IIa 52 GBM patients	500 mg in combination with TMZ/RT→TMZ	Newly diagnosed GBM	Median PFS 8 months (CI 6–11) PFS at 6 or 12 mo: 69% (CI 54–80); 33% (CI 21–46) Median OS 16.1 months (CI 13–23) Survival at 2 years: 35% (CI 22–48)

^a Cilengitide dose administered twice weekly i.v. over 60 min

DLT dose-limiting toxicity, MTD maximally tolerated dose, CR complete response, PR partial response, SD stable disease, GBM glioblastoma, AA anaplastic astrocytoma, PNET primitive neuroectodermal tumor, CI 95% confidence interval, PFS progression-free survival, PFS-6 progression-free survival rate at 6 months, OS overall survival, TMZ/RT→TMZ concomitant chemoradiotherapy with temozolomide, followed by adjuvant temozolomide [46], NABTT New Approaches to Brain Tumor Therapy, NABTC North American Brain Tumor Consortium, PBTC Pediatric Brain Tumor Consortium, EMD Merck Serono

treated with the higher dose of cilengitide [42]. Similarly, a phase II randomized trial evaluating lower (500 mg) and higher (2000 mg) doses cilengitide added to temozolomide and radiotherapy (TMZ/RT) in newly diagnosed glioblastoma patients was reported in abstract form [43]. Median survival was 18.9 months, with an estimated median survival of 17 months and 21 months, for the lower and higher dose, respectively. Finally, higher exposure of the tumor tissue could be demonstrated in another randomized trial evaluating 3 doses of either 500 mg or 2000 mg cilengitide prior to tumor resection in recurrent disease. Cilengitide concentrations were several-fold higher in the tumor than the corresponding plasma concentrations, demonstrating both excellent penetration through the blood-tumor barrier and retention within the tumor. Importantly, safety could be confirmed and no increase in bleeding complications or wound healing problems were observed [44].

Consistently, the higher dose of cilengitide is favored by exposure, responses, progression-free and overall survival.

Based on a strong preclinical rationale of radiosensitization and synergy with both cilengitide and irradiation and TMZ, we designed in parallel to the above-mentioned trials a phase II pilot trial of adding cilengitide (500 mg) to standard chemoradiotherapy with TMZ (TMZ/RT→TMZ) [45, 46].

In a multicenter pilot study on 52 patients, treatment was well tolerated with no identifiable added toxicity. The primary endpoint of progression-free survival at 6 months compared favorably with historical controls (69% versus 54%) [46, 47]. Median survival was 16.1 months, with a 2-year survival rate of 35% [45]. Interestingly, the outcome was particularly good in patients with a methylated O⁶-methylguanine–DNA methyltransferase (*MGMT*) gene promoter. Such patients were previously identified to particularly benefit from TMZ chemotherapy [48]. This is consistent with the working hypothesis, that antiangiogenic therapy may lead to normalization of tumor vasculature, decrease of hypoxia, improved drug delivery and enhanced cytotoxicity. In addition, the currently used higher dose of 2000 mg should also exert relevant direct antitumor activity and directly synergize with standard chemoradiotherapy.

Building on our pilot experience and the consistent encouraging results of the other clinical trials in malignant glioma, Merck KGaA in collaboration with the European Organisation for Research and Treatment of Cancer (EORTC) and the Canadian Brain Tumor Consortium is sponsoring a large prospective clinical phase III trial (CENTRIC study, Fig. 1) [49]. Based on the suggested synergy between normalized perfusion and active chemotherapy, only patients with a methylated *MGMT* are eligible.

This requires centralized upfront *MGMT* testing, and it is the first prospective international clinical oncology trial incorporating this novel molecular marker to homogeneously define the treated patient population. Other changes from the previous phase II trial are the exclusive use of the higher dose of cilengitide (2000 mg), and cilengitide maintenance therapy for up to 18 months. In parallel a separate 3-arm randomized phase II trial has been designed for patients with *MGMT* unmethylated tumors (CORE study). Here an intensified high-dose daily cilengitide administration before radiotherapy is being explored. Early data indicate that this schedule is well tolerated and safe, and the trial is now ongoing in a multicenter setting. The results of all completed brain tumor clinical trials using cilengitide are summarized in Table 1.

Conclusions

The ongoing phase III CENTRIC trial is successfully recruiting in a multi-national setting and is expected to be completed by the end of the year 2010. Careful analysis of data emerging from this trial will make an important contribution to further defining the role on integrin inhibition in malignant gliomas. The absence of significant toxicity and excellent tolerance will allow to safely testing integrin-inhibition in combination with other targeted and cytotoxic agents. Combined inhibition of integrins and VEGF may lead to further enhancement and increased antitumor effect, and clinical evaluation of this concept is warranted.

Conflict of interest statement The authors have no significant conflict of interest to declare. The paper was written by G. Tabatabai and R. Stupp, with review, input, comments and final approval by all authors. The authors have conducted and are conducting industry and/or NCI-sponsored trials with cilengitide.

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