Published in final edited form as:

J Child Neurol. 2008 October ; 23(10): 1214–1220. doi:10.1177/0883073808321771.

Targeting Hyaluronan Interactions in Spinal Cord Astrocytomas and Diffuse Pontine Gliomas

Bernard L. Maria, MD, MBA, Nalin Gupta, MD, PhD, Anne G. Gilg, PhD, May Abdel-Wahab, MD, Anthony P. Leonard, BS, Mark Slomiany, PhD, William G. Wheeler, BS, Lauren B. Tolliver, MS, Michael A. Babcock, BS, John T. Lucas Jr, BS, and Bryan P. Toole, PhD
Departments of Pediatrics (BLM, AGG, WGW, LBT), Neurosciences (BLM), Cell Biology and Anatomy (MS, BPT), Charles P. Darby Children's Research Institute, College of Medicine, Medical University of South Carolina, Charleston, South Carolina (APL, MAB, JTL); the Department of Neurosurgery, University of California, San Francisco (NG); and the Department of Radiation Oncology, University of Miami (AWM)

Abstract

Although significant advances have been made in treating malignant pediatric central nervous system tumors such as medulloblastoma, no effective therapy exists for diffuse pontine glioma or intramedullary spinal astrocytoma. Biology of these 2 tumors is poorly understood, in part because diffuse pontine gliomas are not treated surgically, and tumor specimens from intramedullary spinal astrocytomas are rare and minuscule. At the 2007 Neurobiology of Disease in Children Symposium, we presented evidence that malignant glioma behaviors, including antiapoptosis, invasiveness, and treatment resistance, are enhanced by hyaluronan, an extracellular glycosaminoglycan. We review the clinical course of pediatric intramedullary spinal astrocytoma and diffuse pontine glioma, and show expression of membrane proteins that interact with hyaluronan: CD44, extracellular matrix metalloproteinase inducer, and breast cancer resistance protein (BCRP/ABCG2). Furthermore, we describe novel animal models of these tumors for preclinical studies. These findings suggest that hyaluronan antagonism has potential therapeutic value in malignant central nervous system tumors.

Keywords

hyaluronan; glioma; astrocytoma

There are well over 100 histological subtypes of pediatric central nervous system tumors, the behaviors of which depend on heterogeneous genetic and molecular mechanisms, often within the same histological variant. Indeed, histologically similar central nervous system tumors that arise at different ages or from different sites within the nervous system may have a different cellular origin and clinical behavior. This is of primary importance because it most likely affects response to treatment. Moreover, small subpopulations of cells within tumors exhibit stem-like cell characteristics, including self-renewal, multipotency, and high degrees of treatment resistance. Thus, there is enormous complexity in the molecular mechanisms driving malignant behaviors, including antiapoptosis, invasiveness, and resistance to treatment.

During the last quarter century, advances in surgical techniques, adjuvant radiation, and systemic chemotherapy have improved 5-year survival rates from 60% to 65% to as high as 80% to 85% for standard-risk medulloblastoma. However, there have been virtually no advances in the treatment of malignant intramedullary spinal astrocytoma or diffuse pontine glioma.

In response to the dismal prognosis associated with malignant brain tumors, the molecular age has brought a proliferation of novel therapies. These innovations have included novel surgical, radiotherapeutic, and chemotherapeutic strategies, as well as therapies designed to target signaling pathways such as receptor tyrosine kinases and novel oncogenic kinases.^{1,2} However, in the past several years the link between malignancy and treatment resistance has been highlighted in the re-emergence of the cancer stem cell paradigm and the identification of subpopulations of CD133+ stem-like cells that may play a crucial role in resistance to chemotherapeutic agents and radiation therapy.^{3–5} Thus, preclinical testing of new agents is now being refined to determine whether drugs can effectively target these treatment-resistant subpopulations of cells.

Major reasons why current treatments for malignant gliomas fail include these factors: (1) diffuse infiltration of the central nervous system precludes complete resection and (2) residual tumor cells are often resistant to radiotherapy and chemotherapy. Central to the malignant behaviors of glioma cells is their interaction with their environment, including normal glial and neuronal cells, and their surrounding extracellular matrix.⁶ Central nervous system extracellular matrix is highly enriched in hyaluronan, a very large linear glycosaminoglycan. Hyaluronan is distributed ubiquitously in vertebrate tissues but is especially concentrated in regions of high cell division and invasion (eg, during embryonic development and adult tissue regeneration). In a similar fashion to numerous extracellular matrix constituents, hyaluronan serves an important *structural* role, but it also has an *instructive* role in terms of cell signaling via cell surface hyaluronan receptors. It is clear that hyaluronan-induced signaling is “activated” during dynamic cell processes but not under conditions of adult tissue homeostasis.⁷ In particular, hyaluronan is expressed at elevated levels in the pericellular matrices of many tumor types, including gliomas.⁸ Embryonic glial cells produce a larger hyaluronan-based extracellular matrix in culture than more differentiated glial cells.⁹ High levels of hyaluronan in the developing central nervous system and other tissues correspond with stages of active morphogenesis. Glioma cells produce extracellular matrix that is similar in hyaluronan content to that of embryonic brain cells,⁸ and express high levels of the hyaluronan receptor CD44 and receptor for hyaluronan-mediated motility.^{10,11} In addition, malignant human gliomas express high levels of the extracellular matrix metalloproteinase inducer (EMMPRIN).¹² Extracellular matrix metalloproteinase inducer, a cell surface glycoprotein that stimulates both matrix metalloproteinase synthesis¹³ and hyaluronan production,¹⁴ has been shown to induce tumor growth and invasion in vivo.¹⁵

Numerous studies have shown that hyaluronan–receptor interactions are important in glioma invasiveness.^{16,17} Concurrently, our own investigations indicate that antagonists of constitutive hyaluronan interactions strongly inhibit invasiveness and anchorage-independent growth of several glioma cell lines.¹⁸ Hyaluronan also facilitates stem cell migration¹⁹ and may be crucial to the migration/invasion capacity of glioma progenitor cells.²⁰ In addition, hyaluronan may promote drug and radiation resistance in these cells²⁰ (also B. L. Maria, MD, MBA, unpublished data 2008). Taken together, these studies strongly suggest that hyaluronan signaling is “activated” during glioma progression and that hyaluronan may promote invasiveness and resistance to therapy in central nervous system gliomas. Consequently, hyaluronan antagonists that disrupt the signaling processes

supported or initiated by hyaluronan may prove beneficial in therapies for pediatric central nervous system tumors.

To this end, we present the first evidence that human intramedullary spinal astrocytoma and diffuse pontine glioma contain CD44; EMMPRIN, which stimulates hyaluronan production;¹⁴ and the drug transporter breast cancer resistance protein (BCRP/ABCG2), which we previously showed is suppressed by a competitive inhibitor of hyaluronan–CD44 interactions, namely, small hyaluronan oligomers.²⁰ These findings suggest that hyaluronan oligomers hold promise as a new biological therapy for diffuse pontine gliomas, intramedullary spinal astrocytomas, and other malignant central nervous system tumors.

Pediatric Intramedullary Spinal Astrocytoma

It is estimated that fewer than 10% of central nervous system gliomas arise within the spinal cord. These patients often present with back pain, which is otherwise rare in children. Symptoms may also include focal motor deficits, paraparesis, quadriparesis, and bowel or bladder dysfunction, depending on the location of the tumor (Figure 1). Approximately 50% of patients have tumors that extend beyond 5 or more spinal segments. In the largest series of spinal cord gliomas published to date, there were 246 cases reported from 6 institutions.²¹ In this series, there were 22 pediatric astrocytomas and 22 pediatric ependymomas. Although the small numbers could bias survival data, the 5-year, 10-year, and 15-year survival rates for all astrocytomas were only 59%, 53%, and 32%, respectively, despite the fact that two thirds of the astrocytomas were low grade.

Treatment of intramedullary spinal astrocytoma usually consists of surgical extirpation or biopsy with electrophysiological monitoring. Patients with intramedullary spinal astrocytoma are at high risk for spinal cord dysfunction postoperatively and often require large doses of steroids. Malignant astrocytomas are usually treated with adjuvant conformal radiotherapy. However, because these tumors are rare, there are few controlled trials of either radiotherapy or chemotherapy in either high-grade or low-grade intramedullary spinal astrocytoma. Two prospective co-operative group trials have included children with primary spinal cord astrocytomas. In the French Society of Pediatric Oncology trial, 8 children with unresectable or recurrent intramedullary low-grade gliomas were treated with a planned 16-month course of carboplatin, procarbazine, vincristine, cyclophosphamide, etoposide, and cisplatin. Seven of the patients had a clinical or radiographic response to the chemotherapy. Five remained progression-free, with follow-up ranging from 16 to 59 months.²² In the Children's Cancer Group 945 trial, 13 children with high-grade astrocytic spinal cord gliomas were assigned to receive 2 cycles of chemotherapy before radiation therapy, then 8 additional cycles thereafter. At 5 years, 46% of the children had no progression and 54% were alive.²³ Because low-grade astrocytomas often cannot be completely resected from the spinal cord, radiotherapy is often recommended postoperatively, depending on the child's age. Young children diagnosed before their pubertal growth spurt may undergo close follow-up after a subtotal resection if their neurologic function is good, to avoid radiation-induced bone growth delay.

The incidence of malignant transformation with or without adjuvant therapy in intramedullary spinal astrocytoma is unknown. Consequently, there is clearly a need for new therapy that improves outcomes in malignant intramedullary spinal astrocytoma and that enables dose reductions for radiotherapy and chemotherapy, particularly in lower grade pediatric intramedullary spinal astrocytoma. Our analysis of paraffin-embedded intramedullary spinal astrocytoma tissue sections shows that low-grade and high-grade human intramedullary spinal astrocytoma express CD44, EMMPRIN, and BCRP/ABCG2 (Figure 2). It is not yet known whether CD44, EMMPRIN, and BCRP/ABCG2 co-localize

in human intramedullary spinal astrocytoma cells that express the stem cell marker CD133. However, we recently reported that CD133+ C6 rodent glioma cells that were isolated on the basis of their expression of the drug transporter BCRP/ABCG2 also express CD44 and were highly tumorigenic in vivo. Importantly, the growth and invasiveness of tumors that formed from engraftment of parent C6 cells and C6 cells expressing both BCRP/ABCG2 and CD133 were abrogated by hyaluronan oligomers.²⁰

Taken together, these findings suggest that the growth, invasiveness, and treatment-resistance of human intramedullary spinal astrocytoma may also be effectively abrogated by hyaluronan oligomers because tumor cells contain the same cell surface targets for hyaluronan as in C6 glioma cells. In addition, we showed that hyaluronan oligomers sensitize human malignant glioma cells (U87MG) to radiotherapy and chemotherapy in vitro (Figure 3). These cells also express CD44, EMMPRIN, and BCRP/ABCG2 (B. L. Maria, MD, MBA, unpublished data 2008). We also showed that luciferase-transfected U87MG glioma cells could be successfully engrafted into the nude rat spinal cord for preclinical testing of hyaluronan oligomers in combination with irradiation and chemotherapy (Figure 4). In summary, much work needs to be done on intramedullary spinal astrocytoma, which has not been a focus of bench-to-bedside translational research. To date, the evidence suggests that intramedullary spinal astrocytomas grow and invade in a hyaluronan-dependent fashion and that hyaluronan antagonism with oligomers is a promising new treatment modality in targeting glioma cells and treatment-resistant subpopulations of glioma progenitors.

Diffuse Pontine Glioma

Diffuse pontine glioma represents the fourth most common pediatric central nervous system tumor. Children with diffuse pontine glioma develop cranial nerve palsies (most commonly affecting the VIth and VIIth nerves), ataxia, and weakness. The diagnosis of diffuse pontine glioma is often made within 2 to 4 months from onset of symptoms and signs. Magnetic resonance images (MRIs) of diffuse pontine glioma show diffuse enlargement of the pons from tumor growth and edema (Figure 5), with variable degrees of contrast enhancement (often absent), necrosis, and encasement of the basilar artery. Although focal low-grade gliomas (grade 1 pilocytic astrocytoma or grade 2 fibrillary astrocytoma) arise from the tectum, pons, and medulla, their margins are more sharply defined than diffuse pontine glioma, which can often be enhanced with contrast. The typical imaging appearance of diffuse pontine glioma often precludes surgical biopsy prior to treatment. Physiologically, from its pontine epicenter, diffuse pontine glioma rapidly infiltrates the midbrain and thalamus rostrally, the middle cerebellar peduncles, and caudally into the medulla and upper cervical cord. The standard treatment consists of conformal radiotherapy of 54 Gy with concurrent steroid therapy as needed. There is no evidence that high-dose or hyperfractionated radiotherapy, chemotherapy, or immunotherapy is beneficial. During radiotherapy, symptoms and signs usually abate for several months before tumor recurrence within the field of radiation. The prognosis is dismal, with most children showing radiographic and clinical evidence of tumor progression within 1 year of diagnosis. Children with recurrent tumors often experience severe systemic effects of high-dose steroids before succumbing from the disease within 1 year of recurrence. A number of ongoing clinical trials are testing novel agents to improve the life expectancy of children with diffuse pontine glioma (Table 1).

Thanks to Dr Ian Pollack's group at the University of Pittsburgh, we obtained rare paraffin-embedded diffuse pontine glioma tumor sections for analysis. Our analyses of the paraffin-embedded sections showed expression of BCRP/ABCG2 in blood vessels and individual

tumor cells, which also stained for CD133. Extracellular matrix metalloproteinase inducer expression was shown in a membrane-staining pattern (Figure 6).

To test current and novel therapies in malignant brain stem gliomas, luciferase-expressing U87MG glioma cells were successfully engrafted into the nude rat brain stem. There was progressive increase in bioluminescence that corresponded to progressive glioma growth (Figure 7). Although these experiments demonstrate a proof of principle with respect to treatment of animal models of diffuse pontine glioma, more innovative biologic therapies will be required to achieve therapeutic success. In addition, new delivery strategies such as convection-enhanced delivery may be needed to reach effective target concentrations within the pons.

Conclusion

There is evidence that hyaluronan promotes malignant behaviors in glioma cells, including treatment-resistant CD133+ progenitor cells. The fact that human intramedullary spinal astrocytoma and diffuse pontine glioma contain the targets for therapeutic hyaluronan antagonism with hyaluronan oligomers and that treatment-resistant CD133+ subpopulations of human glioma cells co-express membrane proteins that interact with CD44 suggest that antagonizing hyaluronan–CD44 interaction with oligomers may effectively abrogate malignant behaviors in these tumors.

Acknowledgments

This work was supported by a Hollings Cancer Center/Medical University of South Carolina Department of Defense grant titled “Translational Research on Cancer Control and Related Therapy” (Subcontract GC-3319-05-4498CM), by The Malia’s Cord Foundation, by a National Institutes of Health (NIH) Clinical and Translational Sciences Award (BLM, BPT), and by NIH Grants CA073839 and CA082867 and a Charlotte Geyer Foundation Award (BPT). This work was conducted in a facility constructed with support from NIH Grant# C06 RR015455 from the Extramural Research Facilities Program of the National Center for Research Resources. This work also supported by NIH grant 5R13NS040925-09. Presented in part at the Neurobiology of Disease in Children Symposium on Central Nervous System Tumors in conjunction with the 36th annual meeting of the Child Neurology Society, Quebec City, Quebec, October 10, 2007.

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Figure 1. Magnetic resonance imaging of spinal cord glioma. Spinal cord gliomas can occur in the (A) cervical region, (B) thoracic region, or (C) lumbar region. Arrows point to intramedullary tumors.

Figure 2.

Expression of hyaluronan-related proteins in spinal cord gliomas. A, BCRP/ABCG2 (green) and CD133 (red) are co-expressed. B and C, EMMPRIN (green) and nestin (red). Co-localization appears as yellow. D, CD44 (green) is highly expressed. Hoechst dye (blue) labels cell nuclei in (A-D). BCRP, breast cancer resistance protein; EMMPRIN, extracellular matrix metalloproteinase inducer.

Figure 3.

Sensitization of U87MG human glioblastoma cells to radiation and methotrexate treatments by hyaluronan oligomers. A, U87MG cells were irradiated with 0 to 5 Gy $\pm$ hyaluronan oligomers (100 $\mu\text{g}/\text{mL}$ 24-h pretreatment) and then survival was determined by a standard clonogenic assay. Note that addition of hyaluronan oligomers resulted in significantly less colony growth with irradiated cells. B, U87MG cells were cultured as neurospheres to enrich for resistant progenitors. Cells were exposed to increasing concentrations of methotrexate (1–100 μM), a BCRP/ABCG2 substrate $\pm$ hyaluronan oligomers (100 $\mu\text{g}/\text{mL}$). After 48 h, cells were incubated with Cell Proliferation Reagent (WST-1) (Roche, Basel, Switzerland). Results are expressed as percent of control. Note that hyaluronan oligomers treatment sensitized cells to much lower concentrations of methotrexate. BCRP, breast cancer resistance protein.

Figure 4. Tumor growth of U87MG human glioma cells (20 000 cells) transduced with a luciferase-encoding lentivirus was monitored with bioluminescence imaging in the nude rat spinal cord. Control animal received no tumor cells. Positive bioluminescence imaging was detected as early as day 3 and signal followed for 14 days.

Figure 5. Magnetic resonance imaging of brain stem glioma. Note diffuse enlargement of the pons from tumor growth and edema.

Figure 6.

Expression of hyaluronan-related proteins in pontine gliomas. A, BCRP/ABCG2 (green) and CD133 (red). Note high expression of BCRP/ABCG2 (green) surrounding blood vessels and in cells at a distance from blood vessels. B, EMMPRIN (red) is shown expressed at the cell membranes. C, Hoechst dye (blue) labels cell nuclei. Insets enlarged to show detail. BCRP, breast cancer resistance protein; EMMPRIN, extracellular matrix metalloproteinase inducer.

Figure 7.
Brain stem glioma model. Noninvasive bioluminescence imaging of luciferase-expressing U87MG xenografts into the brainstem of nude rat; 100 000 cells were injected.

Table 1**Current Diffuse Pontine Glioma Studies**

Primary trials
Tipifarnib (PBTC-014)
Capecitabine RDT (PBTC-021)
Vandetanib (SJBG07)
Recurrence trials
O ⁶ -benzylguanine and temozolomide (PBTC-015)
Bevacizumab and irinotecan (PBTC-022)
In-design
Bevacizumab, MGMT-directed
temozolomide, and EGFR-directed erlotinib

Abbreviations: EGFR, epidermal growth factor receptor; MGMT, methylguanine-DNA-methyltransferase; PBTC, pediatric brain tumor consortium; RDT, rapidly disintegrating tablets.