Targeting the Tumor Microenvironment to Enhance Pediatric Brain Cancer Treatment

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Abstract: Strategies targeting the microenvironment of pediatric brain cancers have the potential to improve the efficacy of standard and genome-based molecular therapeutics. These strategies also have the potential of helping resolve many of the challenges associated with developing new drugs and running clinical trials for relatively small pediatric brain tumor population. Disrupting vital paracrine and physical interactions between cancer cells and surrounding stroma, targeting and normalizing the abnormal tumor vasculature, and/or inducing antitumor immunity represent some of the most promising approaches. A comprehensive characterization of the pediatric brain tumor microenvironment’s composition and function and its modulation by chemoradiation and molecularly targeted therapies is warranted to develop and effectively implement these approaches.

Key Words: Brain cancers, pediatric brain tumors, pediatric oncology, targeted stromal therapies, tumor microenvironment

Brain tumors are the most common solid cancers in childhood. Over the last decades, overall survival rates in these patients have increased dramatically because of significant improvements in chemoradiation and supportive care, but the outcomes for progressive or relapsed diseases remain dismal. Although these tumors are very heterogeneous in nature, location, and behavior, they are treated with similar therapeutic protocols of standard first-line surgical resection, when possible, followed by rounds of chemoradiation. These regimens are intensive and often cause neurocognitive deficits and endocrine dysfunctions that affect severely the quality of life of long-term survivors. Usually, these children also require prolonged follow-up to monitor for relapse or secondary tumors. Today, one of the outstanding challenges in pediatric neuro-oncology is to provide safer and more comprehensive treatments to minimize or delay craniospinal radiation without compromising existing cure rates.

Recent large-scale genomic profiling of pediatric brain tumors is leading to a fundamental shift in our understanding and classification of these cancers. For instance, extensive genomic mapping has shown that pediatric brain tumors differ significantly from adult tumors, and therefore, it may not be possible to extrapolate directly from experiences of adult protocols and trials. Examples of this are genetic alterations seen commonly in high-grade adult gliomas—EGFR amplification or PTEN deletion—but rarely seen in primary tumors in children. It has also become clear that traditional histological, karyotypic, and clinical classifications largely underestimate the molecular diversity of tumors. In fact, classically defined World Health Organization tumor types represent not a single disease, but a spectrum of distinct diseases with different origins and outcomes, as demonstrated for medulloblastomas, gliomas, and ependymomas.

High-resolution genome sequencing will continue to generate invaluable genetic and epigenetic data to better classify tumors by subtype, to improve risk stratification, and to identify putative new targets and spur the development of new therapies. The possibility of treating each patient according to individually tailored protocols is becoming a remarkable reality and should compel us to reflect on the critical and practical issues of integrating these therapies into the clinic and on how to develop strategies to overcome the main obstacles (Fig. 1).

While major advances have been made in the cancer genomics field, very little is known about the interaction of the pediatric brain tumor cells with their microenvironment. The limited knowledge about the complex tumor-stroma interactions may hamper the successful implementation of genome-based targeted therapies into efficacious treatment protocols. Here, we discuss the most recent stroma-targeted approaches, which we believe could effectively be combined with standard and/or molecular treatment strategies for pediatric cancers.

TARGETING THE MICRONVIRONMENT TO IMPROVE CANCER THERAPY

The inclusion of strategies targeting the tumor microenvironment into current treatments offers a broader and more comprehensive approach to manage cancer. Solid tumors are complex “organs” with an abnormal vascular network and supportive stroma. Cancer cells growing in permanent coevolution with the local microenvironment are heavily dependent on and shaped by multiple paracrine and physical interactions with surrounding cells and matrix components. Targeting the crucial interactions between cancer cells and their microenvironment that contribute to sustaining tumor growth and dissemination and that fuel immunosuppression and resistance to therapies may complement direct targeting of mutant cancer cells and may also help resolve many of the current therapeutic challenges in pediatric oncology.

A major barrier to integrate these therapies is the paucity of data on pediatric tumor microenvironment. The microenvironment of pediatric brain tumors has been far less studied than that of other solid tumors, where the presence and function of stromal components are better understood. In the brain, the tumor stroma is thought to be constituted primarily of neuronal cells, astrocytes, tumor-associated microglia, a matrix with unique composition to promote neural connectivity, and an extensive vasculature, all of which secrete and respond to a myriad of growth factors and cytokines. The nuances of crosstalk between these components and the cancer cells in the context of a developing brain are not fully characterized. For example, the gradual maturation of the matrix and vasculature during postnatal development into adulthood or the nature, function, and routes of recruitment of immune cells to the brain are just now being clarified. Similarly, the role of patient age and...
tumor location is well described with respect to tumor incidence, progression, and survival, but still very little is known about how these factors affect the maturation of stroma.

Although there is a clear need for a detailed characterization of the microenvironment of pediatric brain cancers, targeting this compartment offers obvious advantages. First, stromal cells are genetically more stable than cancer cells and far less likely to develop mechanisms of resistance. Second, targeting stromal factors may help overcome issues related to tumor heterogeneity, because the composition of local brain stroma may be similar and thus may lead to a more straightforward identification of common targets. Finally, a major benefit is the potential simplification of clinical trial design, because aiming at broadly expressed targets should increase the number of eligible patients, reduce costs, and improve cost-effective translation into clinical practice. Over the next sections, we discuss a number of promising and groundbreaking strategies aimed at targeting the stroma and their implications for clinical translation (Fig. 2).

Disrupting Paracrine and Physical Tumor-Stroma Interactions

Cancer cells coopt and exploit their surroundings to survive. These relationships can take the form of paracrine signaling crosstalk, metabolic adaptations, or physical interactions. Strategies disrupting signals between tumor and vasculature\(^1\,^7\,^8\) or the physical interaction between cancer cells and the matrix\(^1\,^9\,^\text{–}\,^2\,^2\) have been proposed. However, these studies need careful preclinical and clinical evaluation. We have recently demonstrated the importance of paracrine loops between cancer and neuronal cells. Specifically, we have shown that medulloblastoma cells depend on close communication with stromal...
granule neurons for survival and invasion. We observed that medulloblastoma cells secrete Shh ligands that induce release of placental growth factor (PIGF), a member of the vascular endothelial growth factor (VEGF) family, by granule cells. Although PIGF is an angiogenic factor, blocking PIGF has modest anti-vascular effects in these tumors. Instead, it signals directly to cancer cells through neuropilin 1 (NRP1) receptor, triggering downstream activation of survival cues conveyed mainly by the mitogen-activated protein kinase pathway. The tumor-host crosstalk circuit of Shh-PIGF-NRP1 signaling is essential for tumor engraftment, growth, and dissemination (Fig. 3). Consequently, blockade of either PIGF or NRP1 with specific clinically tested antibodies leads to a massive regression of primary tumor and decrease in spinal metastatic burden and prolongs survival in 2 human orthotopic xenografts and 1 spontaneous mouse model of medulloblastoma. The most important finding is that PIGF is expressed by 90% of primary medulloblastomas, regardless of their molecular and histological subtype, and high expression of NRP1 correlates with poor overall survival in patients. We have also observed that PIGF is expressed in other pediatric tumors, such as gliomas, ependymomas, and AT/RT, suggesting a therapeutic opportunity in other pediatric brain cancers. Because PIGF has a role in disease but not in normal physiology, and antibodies against PIGF showed a safe profile in adult clinical trials, targeting PIGF/NRP1 may provide an effective treatment option and warrants future evaluation in pediatric clinical trials.

Remarkably similar paracrine brain stroma-tumor interactions were recently found in pediatric high-grade gliomas. In a thought-provoking report, Venkatesh et al demonstrated that neuronal activity stimulates the growth of tumors. The authors showed that neuronal stimulation leads to the secretion of soluble neuroligin 3 (NLGN3) from postsynaptic cells, which in turn activates PI3K/Akt-mTOR proliferative signaling in neighboring glioma cells. Moreover, stromal NLGN3-mediated signaling induces the expression of tumor endogenous NLGN3, reinforcing a feedback loop that promotes tumor growth. In adult glioma patients, levels of NLGN3 mRNA correlate inversely with overall survival. It remains to be seen if NLGN3 equally impacts the outcome of pediatric patients.

The identification of PIGF and NLGN3 as stromal targets in brain cancers raises many fundamental questions for the field. Over the last few years, a considerable research effort has gone into decoding the genomic underpinnings of pediatric tumors. These studies generated invaluable molecular subclassification of tumors, established new guidelines for patient risk stratification, and identified some candidate target genes. However, the translation of these studies to the clinic has been very limited. In medulloblastoma, it has been limited to trials with Shh inhibitors, and it raises 2 general concerns: (i) only 30% of all medulloblastomas show constitutive activation of Shh pathway, and (ii) among those, only a subset has mutations that would be susceptible to the available SMO inhibitors. However, according to our observations, it is possible that Shh has an unexpected larger role in medulloblastoma. We have shown that tumor cells, despite a lack of oncogenic lesions in the Shh pathway, can secrete Shh ligand and rely on this signal for stimulation of PIGF secretion. This suggests that targets showing mutations or amplifications only in restricted tumor subgroups may, in fact, have a broader role than initially anticipated if these “normally” expressed molecules participate in vital tumor-stromal crosstalk. It is also noteworthy that PIGF is expressed in the majority of primary medulloblastomas, even in the absence of Hedgehog genetic lesions. Shh ligands activate paracrine signaling and stimulate the release of PIGF by granule neurons. Stromal PIGF binds to NRP1 receptors on cancer cells and activates downstream mitogen-activated protein kinase signaling that sustains cell survival. Blockade of this signaling axis with specific anti-PIGF or anti-NRP1 antibodies dramatically regresses established primary tumors and spinal metastasis and increases survival. Reproduced with permission from Pollack I, N Engl J Med 2013, 368(20):1942–1943.\[FIGURE 3.\] Tumor-host interactions in pediatric medulloblastomas. Medulloblastomas depend on continuous secretion of PIGF in the microenvironment. Cancer cells secrete Shh ligands, even in the absence of Hedgehog genetic lesions. Shh ligands activate paracrine signaling and stimulate the release of PIGF by granule neurons. Stromal PIGF binds to NRP1 receptors on cancer cells and activates downstream mitogen-activated protein kinase signaling that sustains cell survival. Blockade of this signaling axis with specific anti-PIGF or anti-NRP1 antibodies dramatically regresses established primary tumors and spinal metastasis and increases survival. Reproduced with permission from Pollack I, N Engl J Med 2013, 368(20):1942–1943.
The majority of medulloblastomas across subgroup classification, and NRPI is a potential biomarker of tumor prognosis, but neither protein was uncovered by previous genetic screenings. Likewise, somatic mutations in NLGN3 are very rare in pediatric tumors. This indicates that we may be bypassing detection of many crucial tumorigenic factors, particularly if they do not contain overt genetic lesions or if there is not enough effort put into understanding further the biology of tumor-stroma interactions and the role and prevalence of nongenetic mechanisms of local signal dysregulation. The fact that PI GF, regarded as an angiogenic factor, primarily has direct effects on the tumor cells underscores that the effects of growth factors and cytokines are pleiotropic and largely uncharacterized across cellular contexts. Finally, the fact that neuronal guidance receptors such as NRPI and synaptic cues such as NLGN3 have a prominent role in pediatric brain tumors reinforces the concept of pediatric cancers as developmental diseases that fail to appropriately shut down/regulate normal developmental cues. In support of this observation, we have also seen that the levels of PI GF decrease with normal cerebellar development. Clarifying the regulation of these cues should help explain peak ages or specific locations of tumors and can provide additional potential targets for clinical translation. Given the large molecular heterogeneity of pediatric brain tumors, any stroma-directed treatment protocol must be tailored to specific tumor types and/or patients. Leveraging the large resource of genomic data might also uncover potential tumor stromal targets.

**Targeting the Vasculature—Revisiting Tumor Angiogenesis**

The Food and Drug Administration approval of bevacizumab, a monoclonal antibody against VEGF, for recurrent adult glioblastoma (GBM) prompted the idea of implementing vascular-targeting agents to treat pediatric brain tumors, which also display an abnormal vasculature. Since then, bevacizumab has been given to pediatric patients alone or in combination with conventional drugs in recurrent and progressive cancer settings. Yet, results from small retrospective uncontrolled studies showed conflicting data in terms of its efficacy. Subsequent phase II trials concluded that bevacizumab was well tolerated but had minimal effects in recurrent malignant glioma, brainstem glioma, or recurrent ependymoma. In fact, blocking VEGF alone failed to show prolonged overall survival in any malignant adult or pediatric brain cancer trial. Clearly, antiangiogenesis strategies need to be revisited, and additional studies are warranted to further evaluate the full potential of anti-VEGF therapy against pediatric brain tumors.

One strategy to improve antiangiogenic therapy aims to overcome resistance to anti-VEGF therapies. Up-regulation of proangiogenic molecules, such as angiopoietin 2 (Ang2), is a potential resistance mechanism to anti-VEGF therapy. Preclinical studies, the combined blockade of VEGF and Ang-2 showed significantly improved antitumor effects compared with anti-VEGF therapy alone. Several companies are currently investigating anti-VEGF/Ang2 therapy in clinical trials against solid malignancies, including adult brain tumors (NCT01609790, NCT01248949, NCT01290263). Interestingly, no additional toxicities have been observed in adult patients treated with anti-VEGF/Ang2 therapy compared with anti-VEGF therapy alone, which gives hope that dual VEGF/Ang2 blockade may also be safely used in the pediatric population.

Another important finding, which has emerged from previous preclinical and clinical trials, is that the dose of antiangiogenic therapies matters. We have shown that high-dose anti-VEGF therapy may lead to excessive vessel pruning and increase tumor hypoxia, which is known to reduce the efficacy of chemoradiation, increase tumor cell invasion, and suppress antitumor immunity. In contrast, a low dose of anti-VEGF therapy may actually lead to vessel normalization, reduction of hypoxia, improved drug delivery, and improved immune function. Interestingly, in 2 retrospective studies of more than 200 adult GBM patients, those who received a lower dose of bevacizumab survived longer than did those on higher dose. These preclinical and clinical findings highlight the importance of choosing the right dose of antiangiogenic agents when treating pediatric brain tumor patients.

Another approach to improving the outcome of antiangiogenic therapy is informed and stringent inclusion of patients into clinical trials. The key to implement this successfully is to identify and validate predictive biomarkers of therapy response. This is important not only to improve survival in selected patients but also to spare toxic effects in the rest. Several specific tumor gene signatures have been proposed to predict patient response to antiangiogenic therapy. However, to date, no predictive gene signature has been validated. In line with our preclinical observations described above, we have shown that patients with a normalized tumor vasculature after anti-VEGF therapy may benefit most from antiangiogenic therapy. In fact, we have shown that GBM patients who had an increase in tumor vessel perfusion after anti-VEGF treatment had prolonged survival compared with patients, who had no change or had a reduction in perfusion. Conversely, we found that patients with elevated plasma levels of soluble VEGF receptor 1 are intrinsically resistant to anti-VEGF therapies. Based on the experience in adult tumor patients, it has become clear that significant effort is needed to validate biomarkers, which may help identify pediatric patients likely to benefit from antiangiogenic therapy.

**Normalizing the Microenvironment to Enhance Antitumor Immunity**

Until recently, the brain was thought to be an immune privileged site. The presence of the blood-brain barrier (BBB), the absence of a lymphatic drainage system, and the reduced expression of major histocompatibility complex molecules all contribute to a lack of immune surveillance in the brain. However, this traditional view is currently being challenged by accumulating evidence showing active patrolling of the central nervous system by immune cells. In fact, peripheral immune cells, such as perivascular macrophages and meningeal dendritic cells, are positioned between the blood and brain and continuously monitor the microenvironment for any foreign invasion. The brain resident immune population comprising resting microglia and neuroectoderm-derived astrocytes that maintain central nervous system homeostasis also plays a vital role in initiating immune responses. Therefore, immunological surveillance in the brain is a dynamic, organ-specific and tightly regulated process in order to maintain homeostasis and integrity. During tumor progression, the BBB loses integrity because of local changes in vasculature. Under this condition of enhanced permeability, different subsets of T cells and myeloid cells can migrate to the brain. In fact, the brain tumor microenvironment contains different immune cell populations with protumor (e.g., T-regulatory cells [Tregs], M-2 macrophages) and antitumor (e.g., cytolytic T cells, M-1 macrophages) phenotypes. The presence of antitumor immune infiltrates in tumors associates with better prognosis in patients with brain tumors. Recently, it was shown that different pediatric brain tumors exhibit distinct immune signatures. While pilocytic astrocytomas and ependymomas have antitumor immune cell infiltrates, medulloblastomas show scarce infiltrate and highly immunosuppressed...
phenotype.64,65 As so, in murine models of medulloblastoma, the genetic ablation of transforming growth factor β in T cells to eliminate the functional Treg population induces a shift toward CDS⁺-imparted antitumor immunity and leads to prolonged survival.66 Together, these findings stress the need to develop immunotherapies against pediatric brain cancers that are tailored to tumor type.

Anticancer immune strategies fall into 3 major categories: (i) cell-based therapies, (ii) immunomodulation (e.g., checkpoint blockers—antibodies that block immune suppression), and (iii) passive immunotherapy, which targets cancer cells directly and prevents signaling or secretion of growth/angiogenic factors. Each of these strategies presents different opportunities and challenges in the context of brain tumors. Successful application of these strategies will require an in-depth understanding of the immune local cellular populations, modulation of biological and physical stromal barriers to recruitment, trafficking and function of immune cells and immunomodulatory drugs, and an informed assessment of risks and benefits of such therapies.

Cell-based therapies in pediatric brain cancers have been limited so far to cell-based vaccines.67–71 However, the remarkable success of chimeric antigen receptor (CAR) T-cell therapies against pediatric B-cell lymphoblastic leukemias and lymphomas72 is prompting the use of similar therapies in solid tumors. Clinical trials of CAR-T cells in brain cancers are currently limited to adult gliomas expressing the EGFRVIII variant. The experience gained with the adult trials will be invaluable to future studies in pediatrics. Applying T-cell engineering strategies to brain cancer patients will require not only the identification of appropriate tumor and/or stroma-specific antigens, but also the effective transport of these cells through the BBB and tumor vasculature and the effective function in the local tumor milieu. Next-generation CAR-T cells, which can incorporate multiple antigen recognition domains or express enzymes or cytokines, will expand the range of opportunities to treat hard-to-reach cancers. In the same way, rational combination regimens, using existing therapies that can increase antigen repertoire (e.g., radiation), normalize the environment, or improve cell delivery (e.g., antiangiogenic drugs), are potentially valuable but will need to be rigorously defined before being translated to the pediatric population.

Preclinical studies of immunomodulation in adult gliomas have shown that antibodies against IDO, cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) or programmed death ligand 1 (PD-L1) can decrease Tregs and prolong survival because of enhanced T-cell–mediated immunity.73 In view of that, several antibodies to alleviate T-cell suppression are now in trial. These encouraging results have also prompted the translation of these approaches to pediatric tumors and, currently, pembrolizumab, an antibody against programmed cell death 1 (PD-1) receptor is being evaluated in children with refractory/refractory high-grade or diffuse intrinsic pontine gliomas. Similarly to what was speculated for cell-based therapies, radiation might also be combined advantageously with immunomodulators. The inclusion of radiation in checkpoint blockade therapies or in combination with stimulation of T-cell costimulatory receptors (CD137) was shown to promote survival mediated by CDS⁺ T cells.74,75 The overall immunoprivileged status of pediatric brain tumors is, perhaps, the major barrier to translate these strategies. Effective translation will require detailed identification of resident tumor and immune populations that express immunomodulatory molecules and the availability or recruitment of effector lymphocytes. Effects of these therapies in children without fully developed lymphoid organs remain a question.

One factor that might impede the efficacy of immunotherapy is the abundance of proangiogenic factors that may directly and indirectly contribute to the immunosuppressive tumor microenvironment.14,44,45,76 For instance, VEGF, basic fibroblast growth factor, and endothelin 1 all suppress the expression of various adhesion molecules, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, and the lack of these molecules leads to a reduction of leukocyte recruitment.70–72 Furthermore, the abnormal tumor vessel endothelium preferentially supports migration of immunosuppressive cells, such as Tregs.73,74 These proangiogenic factors inhibit not only the recruitment of immune cells, but also normal immune cell function within the tumor microenvironment.10,75 As an example, VEGF-A affects the maturation of dendritic cells, which when cultured with T cells enhance expression of Treg markers such as interleukin 10, VEGF, CD25, and CTLA-4.75–77 A recent preclinical study indicates that VEGF also regulates the expression of PD-1 on tumor-infiltrating T cells and contributes to immunosuppression.86–88 Thus, targeting VEGF seems a reasonable approach to improve antitumor immunity. Passive immunotherapy using antibodies targeting angiogenic or growth factors can therefore be an attractive option to treat pediatric brain cancers. Indeed, we have shown that judicious use of antiangiogenic therapy leads to vessel normalization resulting in improved tumor perfusion, reduced hypoxia, and improved survival from immunotherapies.55 Along these lines, a recent phase I study has demonstrated improved response rates in metastatic melanoma patients treated with bevacizumab and anti–CTLA-4 antibody.89,90 Whether combining antiangiogenic agents with various immunotherapies can prolong survival in pediatric brain cancer patients is unknown. Using these strategies will require a better understanding of the immune microenvironment of pediatric tumors.

PERSPECTIVE

Therapies for pediatric brain cancers are progressing from conventional regimens to precision medicine protocols. Unparalleled advances in cancer genomics are allowing this transition and with time will undoubtedly uncover a number of effective molecular targets. Genomic approaches are, however, associated with many therapeutic and economic challenges. Targeting the tumor microenvironment is a valuable and undertapped strategy to help treat cancer and address some of these challenges. Disrupting vital paracrine signaling between cancer cells and stromal cells and matrix, targeting the tumor vasculature, or triggering potent and lasting immune responses are some of the most promising current stromal-directed therapies. Inclusion of these therapies into conventional and/or molecularly targeted treatments can have a profound impact for the management of pediatric patients. To develop and use these therapies effectively, 2 main points need to be addressed: (1) characterization of the components and biology of the pediatric brain tumor microenvironment and (2) discovering new strategies to modulate the microenvironment to enhance the outcome of conventional and targeted therapies. The in-depth analysis of the pediatric tumor microenvironment’s composition, function, and dynamics is a prerequisite to realizing these goals.

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