Neurobiology of Disease xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Hypoxia in astrocytic tumors and implications for therapy

David A. Cavazos, Andrew J. Brenner *

Cancer Therapy and Research Center, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

ARTICLE INFO

Article history:
Received 26 February 2015
Revised 1 June 2015
Accepted 12 June 2015
Available online xxxx

Keywords:
Glioblastoma multiforme
Astrocytic tumors
Hypoxia
Metabolic derangement
Aerobic glycolysis
TH-302
CXCL12
CXCR7
Pseudopalisading necrosis
Acetate

ABSTRACT

Glioblastoma (GBM, Grade IV astrocytoma) is the most common and most aggressive of the primary malignant brain tumors in adults. Hypoxia is a distinct feature in GBM and plays a significant role in tumor progression, resistance to treatment and poor outcomes. This review considers the effects of hypoxia on astrocytic tumors and the mechanisms that contribute to tumor progression and therapeutic resistance, with a focus on the vascular changes, chemotaxic signaling pathways and metabolic alterations involved.

Contents

Astrocytoma

Primary CNS tumors account for approximately 1.4% of all cancers and 2.4% of all cancer-related deaths (Howlader et al., 2014). In 2015, an estimated 23,180 new cases of primary malignant brain tumors will be diagnosed and 16,570 patients will die from these tumors (Ostrom et al., 2014a, 2014b). While essentially any neuroepithelial cell in the brain can develop into a malignancy, astrocytes are the most common cell of origin for malignant brain neoplasms accounting for approximately two thirds of malignant CNS tumors. Primary brain tumors that develop from astrocytes are called astrocytomas. The World Health Organization (WHO) classifies astrocytomas into four distinct grades (I–IV) on the basis of their microscopic appearance. Collectively, the grade III and IV gliomas are referred to as high grade gliomas, with grade IV referred to as glioblastoma. With the exception of the rare grade 1 (pilocytic) astrocytoma, these tumors are considered incurable and progress in grade and aggressiveness with time from diagnosis. In fact, nearly 90% of astrocytoma present de novo as a glioblastoma. Once transformation to, or diagnosis of glioblastoma occurs, survival is quite poor, and with best available care the median survival is approximately 16 months with a 5 year survival rate of 3.3% (Ostrom et al., 2014a, 2014b).

The poor survival is attributable partly to the nature of the tumor. The infiltrative nature of GBM results in difficulty eliminating microscopic disease despite macroscopic gross-total resection, with 90% of patients having recurrence at the original tumor location (Hou et al., 2006). The location of the tumor also makes drug delivery difficult with only small or lipophilic molecules able to cross the blood brain

* Corresponding author. Fax: +1 210 692 7502.
E-mail address: brennera@uthscsa.edu (A.J. Brenner).
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.06.007
0969-9961/

barrier to reach the tumor. Of those agents that are able to reach the tumor, GBMs have shown to be resistant to most cytotoxic agents and to quickly develop resistance when initially sensitive. Hypoxia plays a significant role in both resistance to treatment and poor outcomes. The impact of hypoxia and potential means to target hypoxia for improved outcomes in astrocytoma is the focus of this review.

The most significant advance in treatment for GBM over the last decade has come from concomitant chemoradiotherapy with temozolomide. While radiation alone is able to produce a median survival of 12.1 months, the addition of temozolomide increases median survival to 14.6 months (Stupp et al., 2005). Just as significantly, approximately 1 in 5 patients is alive in the temozolomide treated population whereas essentially no patients survive to 3 years in the absence of temozolomide. This survival benefit is predominantly driven by epigenetic modification of the methyl guanine methyl transferase (MGMT) promoter, which results in inactivation and inability to repair guanine methylation induced by temozolomide (Hegi et al., 2005). Unfortunately, MGMT silencing only is present in 40% of GBM, suggesting that improvement in treatment by the addition of temozolomide is not realized in the approximately 60% of patients that maintain MGMT expression. No alternative is available in the newly diagnosed setting, and these patients are treated with temozolomide none the less. When combined modality therapy fails, as is universally the case, antiangiogenic treatment with the monoclonal antibody bevacizumab (Avastin) is the mainstay of salvage therapy. The median progression free survival for bevacizumab is approximately 5 months with a median overall survival of 9 months (Friedman et al., 2009). Combination therapy with either irinotecan or lomustine is often considered for patients with good performance status (Taal et al., 2014). Once patients fail salvage therapy, clinical trials are often recommended with no agents proven to impact survival.

Innate tumor hypoxia during tumor development

Hypoxia is a hallmark of GBM, and this is manifest in as the pathognomonic feature of pseudopalisading necrosis (Fig. 1a) and vascular proliferation (Fig. 1b). Pseudopalisading necrosis is the appearance of areas of hypercellularity surrounding areas of necrosis. These areas of hypercellularity have been well characterized and are not the result of increased proliferation. Rather, these areas are intensely hypoxic (Fig. 1c) and have been suggested to represent two-dimensional histologic representations of tumor cells migrating away from a vaso-occlusive event with distorted, degenerating, or thrombosed blood vessels within the center (Brat et al., 2004). These hypoxic foci have high levels of hypoxia induced factor 1 (HIF1) expression, resulting in proangiogenic vascular endothelial growth factor (VEGF) secretion, in turn driving vascular proliferation. However, the formed vessels in response to VEGF are severely malformed, a consequence of perturbation of the normal exquisite counterbalance of antiangiogenic growth factors (Jain, 2013). The result is tortuous and chaotic vessel structure with gaps between endothelial cells and absence of pericytes. Due to malformation and inherent leakiness the interstitial pressure is increased, resulting in vascular stasis with corresponding exacerbation of hypoxia and increased microvascular thrombosis (Jain, 2013). A vicious cycle of vascularization, vascular collapse, and tumor cell migration is repeated which ultimately drives the rapid expansion of cells outward from the tumor margin into the adjacent normal tissue.

As such, intratumoral necrosis and hypoxia are both poor prognostic markers that portend progression and worse survival in patients with GBM (Burger and Green, 1987; Rong et al., 2006). In fact, a developing strategy for the treatment of GBM involves measuring the volume and intensity of intratumoral hypoxia, as determined by fluoromisonidazole positron emission tomography, to quantify and appropriately target cells within hypoxic regions to improve outcome (Spence et al., 2008). Although the underlying pathophysiologic alterations accompanying hypoxia can be understood on a biochemical basis, the impact of cellular adaptation to low levels of oxygen on tumor biology is not fully understood. The long-term sequelae of pseudopalisading necrosis may impart resistance to cytotoxic agents through a variety of mechanisms discussed herein. Because the tumor microenvironment is exceedingly heterogeneous, containing regions of variable oxygenation (Mason et al., 1998), stem cells residing in hypoxic pseudopalisading zones are thought to be partially buffered from the effects of chemoradiation due to vascular stasis and depletion of molecular oxygen. This topic will be further explored in the section Treatment induced hypoxia and resistance. Additional factors that contribute to cancer stem cell drug resistance may include the metabolic reprogramming events associated with GBM. Aerobic glycolysis, also known as the Warburg effect, involves the conversion of glucose to lactate in the presence of molecular oxygen, and is commonly used by normal and malignant cells during periods of sustained rapid proliferation in order to facilitate the synthesis of nutrients into cellular biomass (Hitosugi et al., 2009; Vander Heiden et al., 2011). Despite the inherent inefficiency of glycolysis in generating ATP independent of mitochondrial oxidative phosphorylation (OXPHOS), several groups posit that the intermediates of glycolysis, particularly glyceraldehyde 3-phosphate, are quickly diverted to anabolic pathways as substrate for biosynthesis of the lipids, DNA and protein requisite for rapid cellular replication (Fig. 3). One consequence of this altered glycolytic pathway is that pyruvate is rapidly reduced to lactate and excreted as cellular waste, resulting in less pyruvate dehydrogenase activity and diminished production of acetyl-CoA. Acetyl-CoA is a key metabolic “hub” intermediate where carbohydrate, protein and lipid metabolism pathways converge. Even under conditions of decreased OXPHOS, acetyl-CoA plays a central role in critical metabolic pathways that balance carbohydrate and fat metabolism and is used to biosynthesize nucleotides, amino acids, fatty acids and cholesterol. In nonmalignant cells undergoing aerobic respiration, the majority of acetyl-CoA enters TCA and serves as the carbon source for production of the reducing potential and protons required for ATP synthase activity. Conversely, malignant cells predominantly forego TCA in favor of aerobic glycolysis, resulting in less glucose-derived acetyl-CoA. To compensate, a variety of cancer cell types are known to sequester and metabolize exogenous acetate as an alternative source of generating acetyl-CoA. Acetate is a byproduct of B-oxidation or ethanol metabolism but can be absorbed and conjugated to coenzyme A using one of three mammalian isoforms of short-chain acyl-CoA synthetases (ACSS).

In addition to its importance in energy metabolism, acetyl-CoA also contributes to epigenetic regulation of gene expression and posttranslational protein modification through the reversible addition of acetyl groups onto histones or enzymes (Kaelin and McKnight, 2013). Recent studies demonstrate that human glioma stem-like cells exhibit global hypomethylation and that supplementation with Triacetin, an FDA approved food additive, reduced proliferation in a panel of 6 human primary GBM-derived glioma stem-like cell lines (Long et al., 2015). Parallel studies also show that human GBM cells grown in the brain of mice are capable of capturing and metabolizing exogenous acetate to maintain adequate pools of acetyl-CoA, with as much as 50% being derived from exogenous acetate (Mashimo et al., 2014). Under normal conditions, only a small percent of the acetyl-CoA is produced from scavenged acetate (Lyssiotis and Cantley, 2014). Considering the importance of acetate scavenging in GBM on energy metabolism and epigenetic gene regulation, further studies regarding the role of acetate metabolism are warranted.

It is generally accepted that alterations in energy metabolism may impact the function of cellular organelles involved in drug detoxification. Peroxisomes are intracellular organelles derived from the smooth endoplasmic reticulum that are required for debranching of very long-chain fatty acids prior to L-carnitine mediated transport into the mitochondrial matrix for B-oxidation (Poulos et al., 1992). In the liver, peroxisomes are the site of ethanol detoxification to acetate and water by a series of enzymes including alcohol dehydrogenase, aldehyde dehydrogenase and catalase. In the brain, ethanol is detoxified by cytochrome P450 and catalase. Due to the known perturbations in lipid metabolism

Please cite this article as: Cavazos, D.A., Brenner, A.J., Hypoxia in astrocytic tumors and implications for therapy, Neurobiol. Dis. (2015), http://dx.doi.org/10.1016/j.nbd.2015.06.007