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Bone Marrow-Derived Cells Support Malignant Transformation of Low-Grade Glioma

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1. Introduction

Gliomas, the most common primary brain tumors, exist as a continuum between low-grade and high-grade states. Low grade gliomas are generally found in children and young adults. These tumors are characterized by well-differentiated cellularity which is mildly pleomorphic. These tumors lack mitotic figures and neovascularization and do not enhance on MRI. The average survival of patients after diagnosis is 7-10 years; the morbidity associated with these lesions is largely dependent on progression of these lesions to a higher grade state. High-grade gliomas, conversely, which exist on the other end of the glial neoplasm spectrum, are extremely malignant with poorly differentiated cells that are highly pleomorphic and display numerous mitotic figures. These tumors contain significant vascular proliferation, hemorrhage and necrosis. High grade gliomas enhance brightly on contrast MRI and often exhibit widespread invasion throughout the brain. Prognosis is poor for high grade gliomas, with a median survival of 18 months even with aggressive therapies. One of the key events in the transition from the low-grade to high-grade state has been referred to as the angiogenic switch. This is defined as the period during which the tumor undergoes a transition to an environment capable of rapid blood vessel formation supporting subsequent exponential tumor growth. It is theorized that in the low-grade state, tumor growth may be limited, at least in part, by a lack of blood supply limiting the tumor to linear growth. Once the tumor acquires the ability to recruit or form new blood vessels through this angiogenic switch, exponential growth may occur, which results in rapid clinical progression. It has been well-described in the literature that bone marrow-derived cells (BMDC) participate in the progression of cancer. BMDCs in the local tumor microenvironment have been proposed to be capable of breaking down normal structures thereby promoting vasculogenesis and invasiveness. This, in turn, provides an environment capable of sustaining and promoting tumor growth. The role of BMDC in metastatic disease has been well-documented and recent data suggests that BMDC participate in the growth and progression of brain tumors as well. This chapter will explore the role of BMDC in the transition from low-grade to high-grade gliomas particularly with respect to the angiogenic

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switch. The possibility of this pathway as a potential therapeutic target will also be reviewed.

2. Low-grade glioma transformation in adults

Low-grade gliomas (LGG) are a heterogeneously diverse group of tumors with a generally benign histology and an associated variable outcome. This unpredictable course relies, in part, on the potential for malignant transformation to a higher grade. These tumors present a unique therapeutic challenge as they are typically associated with minimal symptoms and benign radiographic appearance. Initially, the majority of LGGs run an indolent clinical course but often ultimately progress into aggressive tumors with a poor prognosis. As a result, significant controversy exists as to appropriate treatment protocols for this disease. The natural history of LGG, and the risk factors for progression, have been one focus of glioma research due to the potential impact on treatment strategy. Many recent studies have helped clarify treatment recommendations including extent of resection, timing and efficacy of radiation therapy, and response to chemotherapy. Significant debate remains, however, regarding standardization of treatment for low-grade glioma given the tremendous diversity in tumor histology, biology, and outcome. While observation of low-grade gliomas was previously considered a valid treatment option to avoid the morbidity of surgery, chemotherapy, and radiation, early intervention has gradually become standard of care as the impact and incidence of malignant progression has become fully realized. Subjecting patients to the morbidity of aggressive treatment in an unpredictable tumor with variable outcome remains controversial, however. Currently, significant effort is focused on identification of risk factors and tumor characteristics that lead to progression. Better appreciation for the molecular and cellular mechanisms of malignant transformation carries the potential to create novel treatment regimens with less morbidity, thereby alleviating the use of radiation and chemotherapy which present significant toxicity to both children and adults. A review of the characteristics of low-grade gliomas, current treatment strategies, their transformation potential, and current efforts to define novel pathways involved in malignant transformation follows.

The term LGG includes World Health Organization Grade I and Grade II tumors, which are typically associated with indolent tumor growth and significantly better prognosis compared to high grade gliomas. Grade I gliomas include pilocytic astrocytoma, desmoplastic neuroectodermal tumors, subependymoma, ganglioglioma, myxopapillary ependymoma, and desmoplastic infantile tumors, which represent a spectrum of typically benign lesions. Within this class, pilocytic histology is the most common (Stieber, 2001). These pilocytic tumors are well-circumscribed, non-infiltrative, and do not generally transform to more malignant, higher grade lesions. While malignant transformation has been reported in WHOI tumors, the primary risk for malignant degeneration exists in Grade II tumors including low-grade or fibrillary astrocytoma, oligodendroglioma, or mixed oligo-astrocytoma. Ependymoma, ganglioglioma, pleomorphic xanthoastrocytoma, and choroid gliomas of the third ventricle are also considered grade II. Fibrillary astrocytomas, which comprise the majority of grade II lesions (Stieber, 2001), have garnered significant attention due to the significant morbidity and mortality of patients with this diagnosis.

While WHO I gliomas are typically well-circumscribed tumors with benign histology, WHO II gliomas are diffuse, infiltrative and have malignant potential (Stieber, 2001). Both classes, however, are associated with slow tumor growth. The incidence of LGG is reported to be

between 2,700 and 4,700 cases per year, comprising approximately 30% of all malignant gliomas (Schiff et al., 2007, Wessels et al., 2003). These tumors are most common in Caucasian males, and typically present in the second to fourth decades (Schomas et al., 2009, Wessels et al., 2003). Patients greater than 60 years of age carry a poorer prognosis with generally lower Karnofsky scores and larger tumor burden at diagnosis. In adults, the most common presenting symptom is seizure followed by incidental findings on imaging. Less common presentations include trauma trauma, sinus pathology, and pituitary disorder (Wessels et al., 2003). Thirty percent of patients present with neurological deficit, and only 10% present with symptoms of raised intracranial pressure ICP. Speech and language deficits have been reported in 10% of patients (Prabhu et al., 2010), however, focal deficits are less common (Schomas et al., 2009, Wessels et al., 2003).

In adults, LGGs are generally hemispheric, supratentorial, and typically occur in the frontal and temporal lobes. They may involve eloquent cortex, which limits the capacity for gross total resection due to significant risk of morbidity (Prabhu et al., 2010, Stieber, 2001). LGG are hypointense on T1 weighted magnetic resonance imaging (MRI), hyperintense on FLAIR and T2 sequences, and enhance in 30% of cases. There is often associated vasogenic edema (Prabhu et al., 2010, Wessels et al., 2003).

LGG are typically sporadic tumors, although they can occur in association with Li Fraumeni syndrome and Neurofibromatosis Types 1 and 2 (Prabhu et al., 2010, Wessels et al., 2003). Additional risk factors include previous irradiation and exposure to industrial chemicals (Prabhu et al., 2010, Wessels et al., 2003). Allergy has been reported to lower the risk for LGG, suggesting a possible role for immune surveillance in tumor pathogenesis (Prabhu et al., 2010). Survival is highly variable for LGG as median overall survival (OS) is reported to range from 3 to 40 years. Median progression free survival (PFS) is only 50% at 5 years and 17% at 15 years (Bauman et al., 1999, Berger et al., 1994, Jaeckle et al., Stieber, 2001). Median time to progression is 7.2 years (Schomas et al., 2009). In adults, the overall malignant transformation rate ranges from 35-89% with 74% in primary astrocytoma, 70% with mixed tumors, and 45% with primarily oligodendroglial histology (Jaeckle et al., 2010). Importantly, 50% of low risk adults, defined as patients less than 40 years of age with gross total resection (GTR), underwent transformation within 5 years (Jaeckle et al., 2010, Schiff et al., 2007).

Multiple studies have found that age greater than 40 years, extent of resection, tumor diameter greater than 6cm, tumor crossing midline, neurological deficit at diagnosis, and astrocytic histology are risk factors for poor prognosis in LGG (Bauman et al., 1999, E. G. Shaw et al., 2008, Stieber, 2001, Jaeckle et al., 2010, Schiff et al., 2007, E. G. Shaw & Wisoff, 2003). The NCCTG found that astrocytomas carry a worse prognosis than oligodendroglioma. Other retrospective reports corroborate these finding and further specify gemistocytic astrocytoma as carrying a worse prognosis (Jaeckle et al., 2010, Schomas et al., 2009, Stieber, 2001, Wessels et al., 2003, E. G. Shaw et al., 2008). Contrast enhancement, Karnofsky score, mitotic activity, and genetics have also been identified as risk factors for progression (Schiff et al., 2007, Schomas et al., 2009, E. G. Shaw & Wisoff, 2003, Stieber, 2001). Additionally, a Ki67-MIB1 index greater than 4% is associated with a more rapid rate of transformation.

3. Malignant transformation of pediatric low-grade glioma

The presentation and prognosis of LGG in children differs significantly from that in adults. Overall survival and rate of malignant transformation is significantly different in the

pediatric population, leading to the hypothesis that tumor biology in children is inherently different from that in adults. For LGG in children, the overall rate of malignant transformation ranges from 4.3%-38%, which is much lower than in adults (Armstrong et al., 2011, Pollack et al., 1995). This difference may be accounted for in part by the higher rate of pilocytic astrocytomas that comprise the vast majority of pediatric LGG, a histological subset that rarely transforms (Tihan et al., 1999). While no prospective studies have been performed to identify reliable risk factors for transformation in children, radiation therapy is reported to be a possible causative agent (Dirks et al., 1994). Mean time to transformation is relatively short at approximately 6.4 years (Dirks et al., 1994). While the overall rate of progression is certainly lower in children, the risk of transformation in this population is still significant and warrants active and expectant observation.

Despite this risk for malignant degeneration, overall prognosis for children with LGG is significantly better than that for adults. Overall survival in children with LGG 65-90%, however, OS is 51% when pilocytic pathology is excluded (Armstrong et al., 2011, Fisher et al., 2008, Pollack et al., 1995). Following gross total resection, survival is 90-100% with 0% progression, in comparison to the adult transformation rate of 50% even in low risk, young patients with complete resection (Pollack et al., 1995). Progression free survival is between approximately 50% at 10 years, and 53% at 15 years (Armstrong et al., 2011). Gross total resection has been the only factor currently identified to have an impact on progression free survival in children with 0% progression with GTR and 17% progression with near total resection (Pollack et al., 1995). Due to the infiltrative nature of non-pilocytic grade II astrocytomas, this histology in children is more comparable to the adult population and is associated with poorer prognosis (Pollack et al., 1995).

4. Effect of resection and adjuvant therapy on malignant transformation

Currently, initial treatment consists of pharmacologic seizure control if patients present with seizures and steroids for vasogenic edema (Prabhu et al., 2010, Stieber, 2001). For patients with lesions amenable to surgery, the goal is gross total resection as many studies have found overall survival to correlate with extent of initial resection irrespective of adjuvant therapy. At 5 years, OS was 63% with GTR versus 27% OS with STR (Prabhu et al., 2010). Recurrence is also higher with STR (Prabhu et al., 2010). Berger et al. (1994) reported no recurrences within 54 months with GTR, 14.8% recurrence with residual tumor less than $10cm^3$, and 46.2% recurrence with residual greater than $10cm^3$ (Berger et al., 1994, Stieber, 2001). In some cases, tumor location within or near eloquent cortex limits the extent of resection, therefore, newer methods including functional MRI, fiber tracking with diffuser tensor imaging (DTI), intra-operative stimulation and mapping, or intra-operative MRI have helped reduce morbidity and allow more aggressive surgery. As survival decreases with lower Karnofsky score, while the surgical goal remains complete resection, equally important is the avoidance of new neurological deficit (Gil-Robles & Duffau, 2010, Schomas et al., 2009).

The role of adjuvant therapy following surgical resection remains controversial. Although LGG are fairly slow growing tumors with low or absent mitotic activity, their infiltrative behavior and high rate of recurrence and malignant transformation has caused most centers to institute adjuvant therapy regardless of the extent of resection. Recent prospective trials have addressed the role of radiation therapy (E. Shaw et al., 2002). RT was found to improve PFS but not OS (Stieber, 2001). As a result, early RT is administered to patients at high risk

for malignant transformation (defined as age > 40yrs, astrocytic histology, crossing midline, diameter > 6cm, or intractable seizures) or for control of disease at the time of progression (Prabhu et al., 2010). The study also recommended RT to all patients greater than 40 years of age irrespective of resection, as age was the most consistent prognostic factor for malignant transformation (Stieber, 2001). For patients aged 18 to 40, RT was recommended only for patients with incomplete resection. Regardless of these data, treatment protocols vary widely and are often practitioner dependant.

Chemotherapy has also been used as an initial treatment in LGG, most commonly in the setting of unresectable disease, or in patients less than 3 years of age in which RT should be deferred (Prabhu et al., 2010). The response rates to available agents are highly variable, with favorable responses reported between 10 and 60%. Poor response is often associated with low grade tumors as they tend to have lower sensitivity to chemotherapeutic agents due their inherently slow growth and minimal mitosis (Prabhu et al., 2010). The Southwest Oncology Group (SWOG) investigated the use of CCNU in addition to RT following GTR and found no added benefit of CCNU (E. G. Shaw & Wisoff, 2003). The NCCTG found a favorable response using PCV in the treatment of primary disease. Currently, the RTOG is investigating the safety and efficacy of PCV in unfavorable patients following resection and RT. Temozolamide is also under investigation for use in LGG patients at high risk for transformation (Schomas et al., 2009). Clearly, the use of adjuvant therapy requires more investigation before formal recommendations can be defined. Until then, adjuvant therapies will remain controversial and site dependant.

Treatment of pediatric gliomas is subject to a different set of considerations and standards as toxicity of therapy has a greater impact on the developing nervous and skeletal system. Surgery with GTR is the primary mode of therapy as this has been shown to be the most effective method for cure (Fisher et al., 2008, Unal et al., 2008). While rare, malignant transformation does occur so observation is not recommended with lesions that are amenable to surgery. As an exception, optic and hypothalamic gliomas are treated initially with observation and chemotherapy. Due to their slow growth and associated morbidity with surgery or radiotherapy in these locations, conservative management is standard. Ultimately, these tumors are associated with a worse prognosis due to their location and difficulty of surgical intervention in the event of progression. Similarly, first line of therapy for brainstem lesions is observation and potential biopsy only for progression of symptoms or radiographic appearance (Fisher et al., 2008). Based on the 0% progression in the setting of GTR, RT has no role following complete resection in children, as compared to adults (Pollack et al., 1995).

Although standard dose RT (50.4-54 Gy) has been shown to be effective in the pediatric population, RT is deferred in children irrespective of residual tumor burden, recurrence or progression due to the risk of toxicity including endocrine dysfunction, cognitive impairment with decreased memory, lower IQ, attention deficit, cerebrovascular disease, and secondary neoplasms (Fisher et al., 2008, Pollack et al., 1995). Standard dose RT is associated with 34% cognitive dysfunction compared to 8.6% without RT, and 17% endocrine dysfunction compared to 2.9% without RT (Pollack et al., 1995). Overall, the rate of endocrine dysfunction was 10% and cognitive dysfunction was 21%. These findings support the use of repeat surgery and chemotherapy prior to the use of RT for recurrence in children. Chemotherapeutic agents possess significant toxicity as well. While carboplatin and vincristine showed good response rates with 68% 3 year PFS, 40% of patients demonstrated hypersensitivity reactions. CCNU, vincristine, and dibromodulcitol have all

been associated with significant hypersensitivity reactions (Fisher et al., 2008). As a result, TPCV is now being tested for efficacy and safety in a prospective pediatric trial (Fisher et al., 2008).

5. Histology of malignant transformation

As mentioned previously, low grade gliomas comprise a histologically diverse group of tumors. The current WHO classification describes four categories for astrocytomas (Kleihues et al., 1995, Louis et al., 2007). While it is theorized that the majority of grade IV glioblastomas (GBM) occur *de novo* (primary GBM), a significant number of lesions result from progression of a low-grade tumor (secondary GBM). Excluding Grade I pilocytic astrocytomas as they rarely progress, low grade (II) and high grade (III and IV) astrocytomas can be viewed to exist along a continuum based on the histological analysis of tumor tissue. Grade II lesions are defined by low or absent mitotic activity and, unlike Grade I gliomas, are infiltrative and invasive and should not be considered benign. Cellular density is low to moderate, and well-differentiated, mildly pleomorphic tumor cells are present. One important feature of low grade astrocytomas is the absence of neovascularization.

This is in distinct comparison to high grade gliomas, grade III anaplastic tumors and grade IV GBMs, which are poorly differentiated, widely infiltrative and display prominent mitotic activity and neovascularization. Both confer a poor prognosis. High-grade lesions display increased cellularity, marked pleomorphism and nuclear atypia and may include multinucleated giant cells. Necrosis is the defining feature of GBM and these areas are typically surrounded by pseudopalisading cells. Most importantly, extensive irregular vascular proliferation is present in GBM as these tumors have adopted the capability of undergoing the angiogenic switch to produce their own vasculature, allowing for exponential tumor growth.

While morbidity is associated with low-grade astrocytomas themselves, it is hypothesized that the majority of morbidity is caused by progression to high-grade tumor. One of the key factors in this progression is the angiogenic switch whereby the tumor adopts the ability to acquire its own vascular supply. This enables explosive growth and precipitates rapid clinical deterioration. While an increased understanding of LGG biology and behavior has led to a more aggressive approach to these tumors, clinical outcome measures still remain poor. This is due mostly in part to our inability to prevent or detect malignant degeneration. A significant amount of research is now focused on understanding the factors involved in the angiogenic switch, which is likely to lead to additional treatment targets and potentially better outcomes. This will be further discussed in the sections to follow.

6. Molecular biology of malignant transformation

While histological characteristics currently determine tumor grade in astrocytoma, important molecular differences also exist between low grade and high-grade gliomas (Table 1) (Godard et al., 2003). These molecular differences are likely to be an important factor in initiating or promoting the angiogenic switch (Wen & Kesari, 2008). Both primary and secondary GBM exhibit elevated VEGF expression and loss of heterozygosity at 10q. The majority of primary GBM show overexpression of EGFR and PTEN mutations. In particular, glioblastomas that express the EGFRvIII genetic variant have a worse prognosis

and show resistance to current therapeutic regimens (Furnari et al., 2007, Hatanpaa et al., Johns et al., 2007, Pelloski et al., 2007). While PTEN mutations occur more frequently in primary glioblastoma in adults, PTEN mutations exist in high frequency in pediatric gliomas that have undergone malignant transformation (Broniscer et al., 2007).

Genetic Mutation	Incidence in Grade II Astrocytoma and Secondary GBM	Incidence in Primary GBM
p53 (TP53)	↑↑↑	↑
EGFR	↑	↑↑↑
PTEN	↑↑	↑↑↑
IDH 1&2	↑↑↑	↑↑
PDGF	↑↑↑	↑↑
BRAF	↑	--

Table 1. Various genetic mutations associated with gliomas. EGFR - epidermal growth factor receptor, PTEN - phosphatase and tensin homolog, IDH - isocitrate deyhdrogenase, PDGFR – platelet derived growth factor receptor.

In contrast, secondary GBMs often have p53 mutations and overexpress PDGF. Mutations of p53 frequently are associated with low-grade gliomas occurring in 53% of astrocytoma, 44% of oligoastrocytoma, and 13% of oligodendroglioma (Okamoto et al., 2004). Therefore, p53 may be an important molecular event involved in the malignant progression of low-grade gliomas (Louis et al., 2007). Interestingly, in children, the rate of p53 mutations is reported as only 10% in progressive pediatric LGG. While this alteration may seem to possibly explain the improved survival in pediatric gliomas, the 1p19q deletion, an indicator of a favorable response to specific chemotherapies in adults, is not found in pediatric gliomas (Fisher et al., 2008).

Other molecular changes have also been identified (Ichimura et al., 2009, Watanabe et al., 2009). IDH1 abnormalities exist in 59-88% of diffuse astrocytomas, 68-82% of oligodendrogliomas, 50-78% of anaplastic astrocytomas, 49-75% of anaplastic oligodendrogliomas, and 50-88% of secondary glioblastomas and often co-exist with p53 mutated lesions or 1p19q co-deleted tumors (Hartmann et al., 2009, Ichimura et al., 2009, Parsons et al., 2008, Sanson et al., 2009, Watanabe et al., 2009, Yan et al., 2009). While the presence of IDH mutations in low-grade tumors and secondary GBMs suggests a role for IDH in malignant progression , the literature suggests that the presence of IDH1 or IDH2 mutations correlates with better outcomes in patients (De Carli et al., 2009, Yan et al., 2009). PDGFR and the p16ink4a /RB1 pathway have also been implicated in gliomagenesis as hypermethylation of the RB1 gene may result in uncontrolled cell cycle progression, which may then drive tumor formation (Sathornsumetee et al., 2007). Both primary and secondary GBMs express PDGF, but increased RB1 gene promoter methylation appears to occur more frequently in secondary GBMs (43%) than primary GBMs (14%) (Nakamura et al., 2001).

The expression of MGMT, a DNA repair enzyme, has also been implicated in glioblastoma and low-grade gliomas (Bourne & Schiff, 2010). Of particular interest is the methylation

status of MGMT as it may correlate to resistance to alkylating therapy in some patients (Hegi et al., 2005).

Finally, chromosomal e 7 (7q34) gene BRAF mutations and overexpression of B-raf, which stimulates the mitogen-activated protein kinase (MAPK) pathway, is a major factor in tumorigenesis of pilocytic astrocytomas (Pfister et al., 2008). This mutation is also present in 23-38% of adult grade II astrocytomas. The role of BRAF mutation in progression to high-grade tumors, however, has yet to be elucidated.

Defining molecular differences amongst glioma subpopulations offers an exciting new dynamic in understanding the behaviors of this highly diverse tumor although much work is required before the variability observed is completely delineated. Already, studies are underway to target tumors at the molecular level in hopes of providing better treatment options (Johns et al., 2007). As it is apparent that the angiogenic switch is important in the progression of low-grade to high-grade glioma, defining the molecular changes that promote this event may offer additional treatment benefits. Animal studies have already shown that preventing the angiogenic switch in other solid tumors reduces tumor growth (Lyden et al., 2001). Therefore, further understanding of how specific molecular changes in tumor cells promote angiogenesis may offer promising new treatment options in gliomas.

7. Advancing imaging of low-grade gliomas

MRI is the initial imaging modality of choice in brain tumors. Low-grade gliomas usually appear as well defined lesions with little mass effect. They have low-signal on T1- and high-signal on T2-weighted imaging - particularly on fluid attenuated inversion recovery (FLAIR) sequences where low-grade gliomas are very hyperintense (Kates et al., 1996). Currently, the absence of gadolinium enhancement is used to differentiate low grade versus high grade glioma (Fig. 2) (Castillo, 1994), however, a significant portion of the low-grade gliomas defined by MRI were found to be high-grade after biopsy (Kondziolka et al., 1993). As a result, MRI is not sensitive enough to definitively diagnose low-grade gliomas as there are frequently small areas within the tumor that have already undergone malignant progression. Therefore, advanced imaging technologies, such as perfusion imaging, diffusion-weighted and diffusion tensor imaging, MR spectroscopy, and position emission tomography (PET), are currently being employed to more accurately identify low-grade versus high-grade gliomas. These modalities provide exciting insight into tumor vascularity, cellularity, metabolism, and proliferation and may prove more effective in differentiating low-grade from high-grade glioma particularly in regions within a given tumor.

Since the degree of vascularity correlates with tumor grade in gliomas, (Daumas-Duport et al., 1997) perfusion MRI and MRI with gradient echo differentiates low-grade versus high-grade gliomas based on relative cerebral blood volume (rCBV) (Boxerman et al., 2006, Law et al., 2003, Law et al., 2004, Shin et al., 2002, Sugahara et al., 1998, Sugahara et al., 2001). While promising, it has been difficult to establish a reliable threshold based on rCBV for low- versus high-grade state. Diffusion-weighted MRI has also been utilized based on the apparent diffusion coefficient, which inversely correlates with tumor cellularity (Gauvain et al., 2001, Kono et al., 2001, Sugahara et al., 1999). Again, it has been difficult to reliably predict tumor grade using diffusion MRI (Bulakbasi et al., 2003, Stieber, 2001). Diffusion tensor imaging (DTI) is a modification of diffusion-weighted imaging and measures fractional anisotropy (FA), which correlated with tumor cellularity and vascularity (Price, 2010). DTI is a promising new modality as one study reports the ability to distinguish

between low- and high-grade gliomas using a threshold FA value of 0.188 (Inoue et al., 2005).

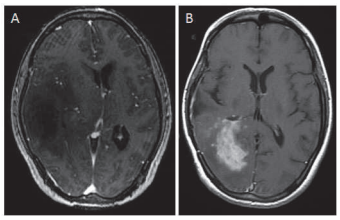

Fig. 1. Serial imaging of malignant progression of glioma. A, T1-weighted MRI with contrast. Patient presented with headache. MRI revealed hypointense lesion in right hemisphere. Note edema and mass effect but lack of contrast enhancement. Pt underwent gross total surgical resection and pathology revealed grade II astrocytoma. B, T1-weighted MRI with contrast. Subsequent imaging revealed recurrent tumor seen as a contrast enhancing lesion in the previous resection cavity. Pathology revealed progression to grade IV astrocytoma (GBM).

MR spectroscopy (MRS) also can potentially differentiate low-grade versus high-grade gliomas in the brain. All gliomas have an increased choline peak and a reduced N-acetyl aspartate peak (NAA) which are markers of membrane turnover and neuronal cell death respectively. Levels of lipid and lactate are markers of necrosis and hypoxia respectively and are decidedly elevated in high-grade compared to low-grade gliomas (McBride et al., 1995, Nafe et al., 2003, Negendank et al., 1996). Creatine (Cr), which serves as a marker of energy metabolism, is decreased in brain tumors (Meyerand et al., 1999, Moller-Hartmann et al., 2002), however, this reduction does not appear to correlate with tumor grade by itself (Moller-Hartmann et al., 2002). Using the choline/Cr ratio may be more effective, however, as low-grade gliomas tend to have a lower ratio of choline/Cr (McBride et al., 1995, Murphy et al., 2002, Sijens & Oudkerk, 2002), as well as an increase in NAA/Cr ratio (Law et al., 2003, McKnight et al., 2002, Murphy et al., 2002, Nafe et al., 2003, Negendank et al., 1996). MRS is a promising technique in differentiating low- from high-grade gliomas with sensitivity between 73% and 92% and specificity between 63% and 100% (Astrakas et al., 2004, Fayed & Modrego, 2005, Law et al., 2003, Nafe et al., 2003, Setzer et al., 2007). MRS may also be capable of identifying regions that have undergone malignant transformation within a given tumor that may not be identifiable by other imaging techniques although one such study attempting to detect malignant transformation within low-grade glioma yielded a specificity of only 57.1% (Alimenti et al., 2007).

Positron emission tomography (PET) imaging has been employed to examine gliomas in the brain by measuring the metabolic activity of tissue. Fluorinated glucose analogue 2-[18F]-fluoro-2-deoxy-D-glucose (FDG), which is administered to patients intravascularly, has high sensitivity for identifying areas of increased tumor metabolism and has been used as an index to predict tumor aggressiveness. While low-grade gliomas tend to have the same or even lower uptake of FDG than normal brain matter, high-grade gliomas demonstrate increased uptake of FDG on PET imaging (Derlon et al., 1997, Tamura et al., 1998), Studies have shown that it is possible to differentiate low- from high-grade gliomas with a sensitivity of 94% and specificity of 77% using a tumor-to-white-matter ratio of greater than 1.5 and tumor-to-grey-matter ratio of greater than 0.6 (Delbeke et al., 1995).

In addition to FDG, other tracers have been utilized in attempts to further characterize these tumors such as carbon 11 and fluorine 18 (18F)-labeled amino acid (Isselbacher, 1972). Methionine PET appears to have a higher sensitivity than FDG PET in detecting low-grade versus high-grade gliomas (Derlon et al., 1997, Giammarile et al., 2004, Ogawa et al., 1993). In particular, methionine PET exhibits a heightened sensitivity in detecting radiation necrosis from recurrent tumors, as inflammatory cells in radiation necrosis have little uptake of methionine (Thiel et al., 2000). Perhaps the most promising technique for diagnosing low-grade gliomas is 18F-FDOPA PET imaging. 18F-FDOPA PET is more accurate that FDG PET and has been shown to be highly predictive in determining tumor grade on initial diagnosis and may help differentiate tumor necrosis from recurrence (Chen et al., 2006, Fueger et al., Tripathi et al., 2009).

While there currently is no one imaging modality capable of definitively determining low-grade from high-grade tumors on its own, advanced imaging technology continues to develop and complement standard MRI. As we come to understand the behavior and variability of these tumors, advance imaging techniques provide exciting new possibilities for more precise treatments. Given the variability within a given tumor, advanced imaging techniques may allow for more precise targets for biopsy, vigilant monitoring of malignant transformation, and improved prognostic power in the management of low- and high-grade gliomas.

8. The Role of bone-marrow derived cells in malignant transformation

The vast majority of brain tumor research, molecular profiling, histological characteristics, diagnostic imaging modalities and treatment targets have focused on the actual tumor cells themselves. As mentioned earlier, one of the key events in the transition from the low-grade to the high-grade state is the angiogenic switch. It is theorized that in the low-grade state, tumor growth may be limited, at least in part, by a lack of blood supply. In this state, the tumor is only capable of a steady-state or linear growth (Mandonnet et al., 2003). Once the tumor acquires the ability to recruit or form new blood vessels, exponential growth occurs (Rees et al., 2009) resulting in rapid clinical decline. While there is considerable evidence that tumor cells undergo continued molecular changes that increase their malignant potential, these changes also allow these cells to initiate the angiogenic switch. It must also be noted that while recent evidence suggests that tumor cells may be capable of directly forming new blood vessels (Ricci-Vitiani et al., Wang et al.), a considerable body of evidence suggests that tumor cells do not do this completely on their own. While the exact details of this process still remain to be fully elucidated, tumor cells acquire the ability to transition the local tumor niche to an environment capable of rapid blood vessel formation. A variety of growth factors, signaling pathways, and indigenous populations of cells is hypothesized to participate in this process. If this theory proves to be correct, this population of cells forms an additional therapeutic target that may be as important as the tumor cells themselves. As current therapies directed at neoplastic cells are limited in part to their toxicity, elucidating other potential treatment pathways may further benefit patient outcome.

Neovascularization is a normal process in tissues and the brain during ischemia. In low oxygen states, cells release signals such as VEGF, PDGF, PlGF and HIF-1 that recruit from local existing vessels within the tissue itself (angiogenesis.) In addition, this can activate distant processes that facilitate neovascularization and may even form *de novo* blood vessels

(vasculogenesis). These factors mobilize bone marrow precursor cells which then travel to the site of ischemia via the bloodstream. It is theorized that these cells facilitate vasculogenesis by breaking down existing structures and creating an environment that promotes new blood vessel growth.

Tumors are capable of adopting this machinery to increase growth and invasiveness by activating the angiogenic switch (Bergers & Benjamin, 2003, Rafii & Lyden, 2008). During early tumor development, neoplastic cells rely on existing blood flow and grow in a slow linear fashion (Mandonnet et al., 2003). Once the switch is initiated and neovascularization brings more oxygen and nutrients, tumor cells grow at a much faster rate and tumor size increases significantly (Rees et al., 2009). This initial process is thought to occur mostly by angiogenesis (Kioi, 2010). The release of proteases and proangiogenic factors causes pericytes to detach from existing vessels creating a defect in the extracellular matrix in the environment surrounding the vessel wall (Bergers & Benjamin, 2003). Endothelial cells proliferate locally and sprout outward into the tumor bed creating newly formed blood vessels feeding the tumor. While angiogenesis is an important factor in the angiogenic switch, vasculogenesis and the contribution of BMDC play a critical role as well. For example, when recruitment of BMDC is impaired in an animal model of lymphoma and lung carcinoma, tumor angiogenesis and growth is significantly decreased (Lyden et al., 2001) suggesting that BMDCs contribute significantly to neovascularization and growth in solid tumors.

In metastatic disease, the contribution of BMDC has been well described (Wels et al., 2008). Endothelial (EPCs) and hematopoietic precursor cells (HPC), mesenchymal stem cells (MSC), myeloid-derived suppressor cells (MDSCs), Tie-2 expressing monocytes (TEM) and tumor associated macrophages (TAM) all are mobilized from the bone marrow to future metastatic sites prior to tumor formation. It should be noted that these primitive cells are prominent during embryology and that a significant population of these cells is not present under normal conditions. While the exact role of each cell type has yet to be fully elucidated, their basic function is to break down normal structures and promote vasculogenesis and tumor invasiveness. The net result is a tumor friendly environment capable of sustaining tumor growth. This has been demonstrated experimentally in a murine model of metastatic disease by implanting m-cherry labeled melanoma cells into the flank of mice with GFP-labeled bone marrow and examining the lungs of these animals over time (Kaplan et al., 2005). It was observed that the first cells to arrive in future metastases were not tumor cells, but actual BMDC. This suggested, at least in metastatic disease, that the environment in future metastatic sites is primed by cells from the bone marrow before tumors can begin to grow in these distant areas (Rafii & Lyden, 2008). This also supports the hypothesis by Stephen Paget over 100 years ago that the tumor microenvironment may play as important a role as the tumor cells themselves.

In the brain, the role of BMDC has only recently garnered attention. One of the basic histological differences between low-grade and high-grade gliomas is a lack of neovascularization. Thus, activation of the angiogenic switch is a key element in the transformation of low-grade to high-grade glioma. Two elements are likely to contribute to this process. Genetic changes in tumor cells that occur during progression of disease activate pro-angiogenic factors. This has been observed in human tumor samples whereby genes involved in angiogenesis are upregulated in glioblastoma as compared to low grade astrocytoma (Godard et al., 2003). Kioi et al. also showed in their animal model that release of soluble factors by tumor cells or cells within the tumor microenvironment including

VEGF, FGF and EGF stimulates local angiogenesis (Kioi, 2010). Secondly, hypoxia is an additional critical event in triggering the switch (Kioi, 2010). As tumor size grows and metabolic demand exceeds local perfusion, hypoxic conditions occur. Release of hypoxia inducible factor-1 (HIF-1α) by tumor cells or cells within the hypoxic tumor environment, combined with stromal cell-derived factor-1 (SDF-1) and CXCR-4 receptor activation, mobilizes BMDCs to the tumor site and promotes vasculogenesis in gliomas (Du et al., 2008, Greenfield et al., 2010, Kioi, 2010).

In an attempt to further understand these processes in gliomas, Du et al. utilized an orthotopic model of GBM in mice to demonstrate recruitment of BMDC in gliomas (Du et al., 2008). Based on their results they theorize that hypoxia and the subsequent release of HIF-1α is the key event in tumor progression. Elevation of VEGF, and subsequent SDF-1 release and CXCR-4 receptor activation, mobilizes BMDC and recruits EPC and myeloid cells to the tumor. The net effect tips the balance to a pro-angiogenic state and neovascularization within the tumor bed. Kioi et al also further theorized that radiation treatment may exacerbate the vasculogenesis process and boost eventual tumor recurrence observed in current treatment regimens (Greenfield et al., 2010, Kioi, 2010). The endothelial-mesenchymal transition and MSC have also been described in metastatic disease (Singh & Settleman). MSC exist within the brain and mobilize to the tumor site as well (Hata et al., Kang et al.). The exact roles of these particular BMDC remain elusive and require more study before they are fully delineated.

In our laboratory, we have begun to investigate the correlation of BMDC mobilization and tumor grade in gliomas (unpublished data.) We used a PDGF-driven mouse model of GBM within which tumors develop slowly from low-grade to high-grade. Low-grade tumors have a clear absence of neovascularization and BMDC are not present within these lesions. In high-grade tumors, however, we have observed a profound increase in larger, irregularly shaped, hemorrhagic vessels and a significant population of BMDC exists that is not observed in low grade tumors. In addition, these cells are located near newly forming blood vessels in the perivascular niche. We have also observed that BMDC are mobilized in the bone marrow and are elevated in the peripheral blood of tumor bearing animals versus controls. In addition, a significant difference in this population of cells in the blood exists between low-grade and high-grade animals. While much work is yet to be done before this process is fully elucidated, it appears that the presence of BMDC correlates with tumor grade and the process of neovascularization. Thus, BMDC have a potential role in the angiogenic switch as tumors progress from low-grade to high-grade tumors.

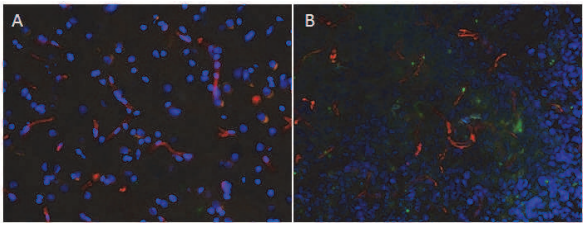

Fig. 2. Bone marrow-derived cells in human glioma. A, Immunofluorescence in grade II astrocytoma shows normal blood vessels (red, VE Cadherin) and a paucity of CD11b+ myeloid suppressor cells (Green). B, GBM shows abnormal vessel formation and an influx of CD11b+ cells (unpublished data.)

Mobilization of BMDC in peripheral blood samples has similarly been observed in patients with astrocytomas. Circulating CD133+ and VEGFR2+ EPC were measured in patients with different grade gliomas. This population of cells was significantly elevated in brain tumor patients versus controls, correlated with tumor grade, and predicted survival. In one patient, this population also predicted recurrence prior to detection by serial radiographic study. Currently, patients are followed with serial imaging in order to diagnose recurrence or malignant progression. While advances in imaging technology show promise in earlier more accurate diagnosis, the critical event has already occurred and prognosis worsens considerably. Therefore, the identification of a potential surrogate biomarker that measures tumor angiogenicity and aggressiveness may potentially serve as an index for ongoing treatment effectiveness or recurrence.

As histological and molecular differences between low-grade and high-grade gliomas are further defined and it becomes apparent that tumors cannot be loosely classified, specific treatments based on the particular characteristics of each individual tumor can potentially be designed. In addition, the presence of particular populations of BMDC in these tumors may also provide additional information on tumor behavior and serve as an additional treatment target along with tumor cells themselves. It has already been shown that the presence of BMDC in the blood correlates with tumor grade and initial animal studies suggest that BMDC are present in high-grade tumors only (Greenfield et al., 2009). In addition, TAM have been associated with poorer prognosis in metastatic lesions and other solid tumors (Wels et al., 2008). Thus histological stains aimed at identifying this population of cells may provide more accurate diagnosis and prognosis. Likewise, the molecular markers of this particular population of cells may offer an even more specific therapeutic target. Based on data collected in glioma patients, EPC can be identified by cell surface markers including CD133 and VEGFR2. Knocking down this population with specifically designed drug therapies has the potential for preventing recurrence by decreasing migration of these cells and reducing vasculogenesis within the tumor bed. Finally, one can also envision a role for advanced imaging technologies for improved diagnosis and treatment. For instance, PET has been used to specifically measure VEGF that has been labeled with copper in an orthotropic mouse model of GBM (Cai et al., 2006). If one could identify and label molecular targets that are specific to individual tumors subtypes and sensitive to new imaging techniques, this provides exciting non-invasive possibilities for tumor specific identification and treatment for each individual patient.

9. Conclusions

In summary, one of the primary factors predicting outcome in patients with low-grade glioma is malignant progression to high-grade tumor and it is evident that the angiogenic switch is an important event in this process. Initial management often entails surgical resection while adjuvant therapy for low-grade gliomas remains a controversial topic. Tumor grade is determined by histological analysis of tumor specimens, but the molecular fingerprint of these tumors is now being analyzed more thoroughly and holds promise for more exciting targeted treatment options. In addition, distinct, but as yet undefined, populations of cells are recruited to the tumor site and participate in neovascularization and promote tumor growth and invasiveness. Therefore, this population may represent an important therapeutic target in combating these tumors. Since survival is directly correlated with tumor grade, preventing tumor progression is imperative. While BMDC certainly are

not the only factor in progression of disease and neovascularization, blocking recruitment of these cells to tumors has been shown to reduce growth in animal models of other tumor types. Therefore, a greater understanding of this process may define a role for targeting this population of cells. In addition, BMDC exist in the periphery, and make for an easier therapeutic target than tumor cells within the blood brain barrier. Lastly, current management of tumor recurrence relies on serial imaging studies. Therefore, an effective and accurate biomarker capable of predicting progression of disease may allow for earlier detection and better treatment outcomes. This makes the case for monitoring BMDC in the periphery in addition to therapy aimed at this population of cells as potential adjuvant therapy in glioma.

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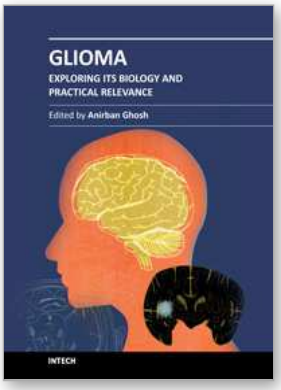

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