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Mini review

Review: Molecular mechanism of microglia stimulated glioblastoma invasion

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ABSTRACT

Glioblastoma multiforme is one of the deadliest human cancers and is characterized by a high degree of microglia and macrophage infiltration. The role of these glioma infiltrating macrophages (GIMs) in disease progression has been the subject of recent investigation. While initially thought to reflect an immune response to the tumor, the balance of evidence clearly suggests GIMs can have potent tumor-tropic functions and assist in glioma cell growth and infiltration into normal brain. In this review, we focus on the evidence for GIMs aiding mediating glioblastoma motility and invasion. We survey the literature for molecular pathways that are involved in paracrine interaction between glioma cells and GIMs and assess which of these might serve as attractive targets for therapeutic intervention.

Contents

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1. Introduction

1.1. Glioblastoma and GIMs

Malignant gliomas are the most common type of brain cancer and arise from glial cells within the central nervous system (CNS). Gliomas that are classified as according to histopathological criteria defined by the World Health Organization (WHO) as grade III and grade IV (also referred to as Glioblastoma multiforme (GBM) (Buckner et al., 2007; Wen and Kesari, 2008; Gladson et al., 2010) are the most aggressive and are characterized by uncontrolled proliferation, areas of necrosis and diffuse infiltration. GBM is one of the deadliest human cancers with a median survival rate of 12 months even with an aggressive treatment consisting of irradiation and chemotherapy (Buckner et al., 2007; Furnari et al., 2007). As therapies for other neoplastic malignancies have made substantial advances over the past several decades, GBM remains essentially untreatable. GBM and high grade astrocytomas are so difficult to treat mainly due to their ability to efficiently infiltrate adjacent healthy brain. As a result of such highly invasive activity, these tumors are unable to be fully resected during surgery. Therefore investigation of the mechanism of glioblastoma cell invasion has received a great deal of interest (Giese et al., 2003; Nakada et al., 2007; Teodorczyk and Martin-Villalba, 2010; Gritsenko et al., 2012; Kwiatkowska and Symons, 2013).

Glioblastoma tumors are heavily infiltrated by cells of myeloid origin, mainly microglia and macrophages. These glioma-infiltrating myeloid cells are collectively referred to here as "GIMs". GIMs can comprise up to 30% of the total tumor mass (Watters et al., 2005). There are recent reviews addressing the role of GIMs in glioma biology (Badie and Schartner, 2001; Watters et al., 2005; Ghosh and Chaudhuri, 2010; Alves et al., 2011; Charles et al., 2012; Li and Graeber, 2012). GIMs have been implicated in playing several roles in GBM progression including proliferation, survival, motility and immunosuppression. The origin of these GIMs seems to be from both resident brain macrophages (microglia) and newly recruited monocyte-derived macrophages from the circulation. The studies which have attempted to distinguish between the two have shown that microglia (as defined by lower CD45 staining) are initially the predominant myeloid cell type to associate with the tumor. However over the course of disease peripheral macrophages are recruited to the tumor in greater numbers (Gabrusiewicz et al., 2011; Tran Thang et al., 2011). Adoptive transfer experiments where donor bone marrow stem cells expressing GFP were transplanted into irradiated recipient animals that were orthotopically injected with a syngeneic murine glioblastoma cell line GL261 that recapitulates most of the features of human glioblastoma disease progression (Miller/Zagzag 2004). This study showed that after three weeks, the vast majority of Iba1 (a marker that specifically labels microglia and macrophages) positive cells at the tumor expressed GFP (Villeneuve et al., 2005). Microglia are the main immune cells of the CNS and are derived from myeloid precursors which migrate into the brain during early embryonic development (Ginhoux et al., 2011; Prinz and Mildner, 2011). Thus far, there is only limited evidence suggesting a differential role for tumor associated macrophages versus microglia (Jacobs et al., 2012a). One study demonstrated that propentofylline (PPF), an atypical methylxanthine with glial modulating and anti-inflammatory properties, selectively interferes with microglial function without affecting macrophages (Jacobs et al., 2012a,b). Using PPF, the authors reported that microglia specifically perform a function that is not compensated by tumor associated macrophages that is critical in establishing a microenvironment conducive to glioma growth.

Macrophages (and presumably microglia) can adopt one of several phenotypes depending on cues from the microenvironment (Martinez et al., 2008; Auffray et al., 2009; Varol et al., 2009; Arima et al., 2010; Biswas and Mantovani, 2010; Yona and Jung, 2010; Biswas et al., 2012; Mantovani et al., 2013). Activated macrophages broadly fall in two categories: so-called "M1" for classically activated, pro-inflammatory and competent antigen presenters versus "M2" for alternatively activated, tumor promoting, and immunosuppressive. This classification scheme however is an oversimplification as there are other types of alternatively activated macrophages described (Mantovani et al., 2009). Tumor associated macrophages typically are shifted toward the M2 side of the spectrum (Sica et al., 2008; Mantovani et al., 2009; Pollard, 2009). A variety of factors secreted by glioma cells have been shown to mediate GIM recruitment and/or conditioning including growth factors, chemokines, cytokines and matrix proteins (Leung et al., 1997; Badie et al., 1999; Hao et al., 2002; Kielian et al., 2002; Kerber et al., 2008; Okada et al., 2009; Held-Feindt et al., 2010; Gabrusiewicz et al., 2011; Yeh et al., 2011; Coniglio et al., 2012; Wang et al., 2012). GIMs are compromised in their ability to promote immune responses however they still retain phagocytic activity (Flugel et al., 1999; Hussain et al., 2006). Further complicating the picture is another type of myeloid cell named Myeloid Derived Suppressor Cell (MDSCs; also referred to as MSCs) which has received recent attention of tumor biologists studying the microenvironment. MDSCs have been implicated in aspects of tumor progression including invasion, angiogenesis and immunosuppression (Serafini et al., 2006; Cheng et al., 2008; Marigo et al., 2008; Corzo et al., 2009; Youn and Gabrilovich, 2011). MDSCs express markers which are found on immature myeloid precursors. It is unclear however if MDSCs are mobilized and recruited directly from the bone marrow or if certain myeloid populations which already exist at the tumor can be reprogrammed towards this state (Rodrigues et al., 2010).

Recent attempts have been made globally ablate microglia and macrophages in order to ascertain the role of GIMs in glioblastoma progression *in vivo* (Galarneau et al., 2007; Markovic et al., 2009; Zhai et al., 2011). These studies attempted to completely ablate GIMs by utilizing transgenic mice which harbor the thymine kinase gene under the transcriptional control of the CD11b (a gene expressed by all mature macrophages) promoter. When gancyclovir is administered to these mice, CD11b expressing cells (i.e. all mature myeloid cells) metabolize it into a toxic end product resulting in cell death. Surprisingly however, three independent reports whereby GL261 cells were orthotopically injected into the CD11b-TK mice yielded somewhat different results. Although the issue of glioma invasion per se (as opposed to general tumor growth) was not directly assessed in these studies, Galarneau et al. demonstrated that ablation of GIMs resulted in an increase in tumor expansion whereas Markovic and Zhai et al. showed a substantial decrease in tumor size. Part of the discrepancy between these results may be explained by the fact that gancyclovir when administered peripherally and there was only a partial reduction (40%) in the number of GIMs in the Galarneau study. However in the other two studies, ganciclovir was administered directly into the brain near the tumor site and nearly 100% removal of all GIMs was achieved. These data suggest GIMs are important for promoting tumor growth and survival however certain subtypes may exist which serve to prevent tumor progression. The preponderance of evidence shows that GIMs are largely pro-tumorigenic with the exceptions suggesting a complex role for GIMs in glioblastoma development. A more subtle treatment of administering the immunosuppressant compound cyclosporin A, which was shown to interfere with GIM function, limited the extent of GBM invasion *in vivo* consistent with what was observed in brain slices. Below we discuss several molecular pathways that have been shown to mediate microglia-stimulation of glioma invasion.

1.2. GIMs and invasion

Studies within the last decade have sought to determine the role of GIMs in motility and invasion. Using standard Boyden chamber assays, it was shown that microglia can substantially enhance the migration of GL261 cells when they were placed in the bottom portion of the chamber (Bettinger et al., 2002). This effect was specific to microglia, as oligodendrocytes or endothelial cells did not influence glioma migration. It was also observed that microglia pretreated with LPS or GMCSF were especially effective at promoting glioma migration. The authors

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