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Mini-review

Mechanisms regulating glioma invasion

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ABSTRACT

Glioblastoma (GBM) is the most aggressive, deadliest, and most common brain malignancy in adults. Despite the advances made in surgical techniques, radiotherapy and chemotherapy, the median survival for GBM patients has remained at a mere 14 months. GBM poses several unique challenges to currently available treatments for the disease. For example, GBM cells have the propensity to aggressively infiltrate/invade into the normal brain tissues and along the vascular tracks, which prevents complete resection of all malignant cells and limits the effect of localized radiotherapy while sparing normal tissue. Although anti-angiogenic treatment exerts anti-edematic effect in GBM, unfortunately, tumors progress with acquired increased invasiveness. Therefore, it is an important task to gain a deeper understanding of the intrinsic and post-treatment invasive phenotypes of GBM in hopes that the gained knowledge would lead to novel GBM treatments that are more effective and less toxic. This review will give an overview of some of the signaling pathways that have been shown to positively and negatively regulate GBM invasion, including, the PI3K/Akt, Wnt, sonic hedgehog-GLI1, and microRNAs. The review will also discuss several approaches to cancer therapies potentially altering GBM invasiveness.

Introduction

Gliomas are primary brain cancers that arise from non-neural cells called glial cells [1]. In the central nervous system (CNS), there are three types of glial cells: astrocytes, oligodendrocytes, and microglial cells. Oligodendrocytes are responsible for myelination while microglial cells are derived from hematopoietic stem cells and phagocytize microbes in the CNS. Astrocytes, the most abundant type of glial cells in the CNS, are star-shaped cells which establish metabolic homeostasis and can shift to a reactive phenotype in response to pathogens or injury in the CNS. This shift is normally a highly regulated process and its dysregulation has been shown to promote malignancy [2,3].

Gliomas can be categorized based on the type of glial cells they are most histologically similar to, the location of the tumor, and the aggressiveness of the cancer cells. Tumors most similar to astrocytes are specifically called astrocytomas and can be further classified into grades I–IV based on the criteria set by World Health Organization, with a higher grade corresponding to more aggressive tumors. Grades I and II astrocytomas correspond to low-grade tumors that are mostly non-malignant. Grades III and IV astrocytomas are high-grade, malignant tumors. Grade III astrocytomas are also known as anaplastic astrocytomas (AAs) while grade IV astrocytomas, commonly referred to as glioblastoma (GBM), are the most aggressive of all gliomas. Unfortunately, GBMs are also the most common type of gliomas with an annual incident rate of 3.19 per 100,000 in the United States [4,5].

While cancer research has made great strides in the treatment of most cancer types, the median survival of patients with GBM is still only approximately 14 months, despite advances in detection, radiation, chemotherapy, and surgery [6,7]. The current standard of care for newly diagnosed GBM patients includes surgery to excise as much of the tumor as safely possible and a combination of radiotherapy with temozolomide (TMZ), an oral alkylating agent which can cross the blood–brain barrier. However, treatment of GBM has remained relatively ineffective because of a number of challenges, including tumor hypoxia which contributes to therapeutic resistance and the invasiveness of GBM tumor cells into normal brain tissues which renders tumor removal insufficient. In particular, the subpopulation of GBM cells with the stem-like self-renewal property has been shown to be highly resistant to various therapies [8]. There is no standard of care for recurrent GBM but one option is the use of a targeted drug called bevacizumab (Genentech). This drug, also known as Avastin, is a monoclonal VEGF-A antibody and is currently the only targeted therapy approved by the FDA to treat recurrent GBM [9]. Development of new drugs has been slow in part because of the ineffective delivery of the drug dosages across the blood–brain barrier and blood–brain tumor barrier. For instance, erlotinib,

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an epidermal growth factor (EGFR) inhibitor, had shown therapeutic promise *in vitro* but failed to show survival benefits in phase II studies because it could not sufficiently cross the blood–brain barrier [10,11].

Since the high degree of infiltration is one of the hallmarks of GBM, this review will summarize the complex, multi-step process of GBM invasion, molecular pathways that have been reported to facilitate GBM invasion, microRNAs that have been associated with the process, and current therapies with the propensity to inhibit GBM infiltration.

Glioma invasion

Even with technological advances in surgical techniques and radiation, malignant gliomas often recur within 1–2 cm of the original tumor site because some of the tumor cells invade into the surrounding normal brain tissue where they can hide from surgical removal and radiation therapy [12]. While other aggressive cancers metastasize by traveling through the circulatory or lymphatic systems to organs, high-grade glioma cells rarely metastasize outside of the brain and instead actively migrate through two types of extracellular space in the brain: (1) the perivascular space that is found around all blood vessels, and (2) the spaces in between the neurons and glial cells which makes up the brain parenchyma and white matter fiber tracts. In order to invade through these spaces, glioma cells typically undergo several biological changes, including gaining mobility, the ability to degrade extracellular matrix (ECM), and the stem cell phenotype.

First, invasive tumor cells become morphologically polarized and develop membrane protrusions allowing the cells to reach forward and attach to the ECM. During this process, invasive glioma cells alter their cell shape and volume in order to move through differently sized spaces, including the extremely small spaces in normal brain [13]. In addition to gaining mobility, invasive glioma cells must be able to interact with multiple components of the ECM. Though the ECM is a physical barrier that glioma cells must get through, it also provides ligands that the tumor cells can anchor to so that they can pull themselves forward. Beyond these physical interactions, the ECM also interacts chemically with glioma cells. Several studies have shown that tumors influence the nearby stromal cells, causing reorganization of the structure and composition of the ECM. These changes in the ECM then further enhance tumor growth and invasion [14]. Cells are inherently motile, but this is tightly regulated in various stages, such as embryological development, and in physiological responses, such as wound healing and immune-response. In glioma cells, motility becomes dysregulated allowing them to be highly migratory [15].

Besides being able to migrate, glioma cells must be able to get through the physical barrier, ECM, by degrading extracellular matrix proteins in order to create a path for invasion. Many studies have reported the involvement of matrix-metalloproteinases (MMPs) in this degradation and the overexpression of several MMPs in cancer cells compared to their normal cell counterparts, including glioma cells [16]. Therefore, it is not surprising that many of the pathways that promote GBM invasion also up-regulate the expression of several MMPs [17–19]. Proteolytic enzymes are tightly associated with invasion. For example, heparanase is an endoglycosidase which degrades and remodels ECM by cleaving heparin sulfate and its overexpression promotes invasiveness of tumor cells *in vivo* [20]. Other proteases implicated in invasiveness include plasmin, cathepsin B, and cathepsin D [21,22].

Any tumor is a heterogeneous population of cells where cancer cells are at different stages of differentiation. Recently substantial attention has been given to a subpopulation of tumor cells called cancer stem cells (CSCs) which like true stem cells are undifferentiated and self-renewing. For gliomas, these CSCs are called glioma stem cells (GSCs) or glioma initiating cells (GICs). GSCs express nestin and CD133, factors associated with neural stem cells, although there are some GSCs that are CD133-negative [23,24]. GSCs also share with normal neural stem cells the ability to form neurospheres in serum-free culture condition, self-renew, and differentiate into different neural cells [25]. GSCs derived from primary human tumors have been shown not only to share many characteristics with neural stem cells, but also to retain the genotype, gene expression pattern, and phenotype of the primary tumor [25]. Because GSCs display more traits of GBM such as excessive invasiveness, this unique cell population is of special interests to GBM research and treatment.

GSCs are considered the primary cause of GBM invasion and recurrence [26]. Cancer stem cells (CSCs) are highly resistant to treatment and if there are CSCs that survive treatment, they are capable of initiating and sustaining new tumor growths, causing tumor recurrences. Therefore these cells are important targets for treatment. Several embryonic signaling pathways, such as Notch, Hedgehog, and Wnt/β-catenin have been reported to help maintain these GSCs and thus provide potential targets for treating these especially malignant cancer cells [5].

Wnt signaling pathway in glioma invasion

Wingless/Int1 (Wnt) signaling regulates many cellular processes in adulthood and plays an important role during embryogenesis [27,28]. Several different intracellular signaling pathways have been identified that can be activated by Wnt ligands and Frizzled (Fz), their seven-transmembrane cell surface receptors. These are divided into those that are dependent on β-catenin and those that are independent (Fig. 1).

The β-catenin-dependent pathway is also known as the canonical Wnt pathway. When this pathway is not activated, β-catenin is bound to its destruction complex which consists of glycogen synthase kinase-3β (GSK-3β), Axin, and adenomatous polyposis coli (APC). GSK-3β phosphorylates β-catenin, marking it for proteasomal degradation. When one of the Wnt factors binds to Fz, it induces Fz to interact with the co-receptor low-density-lipoprotein-related protein 5/6 (LRP5/6), forming a complex that recruits the cytoplasmic scaffolding protein Dishevelled (Ds). This activation eventually prevents GSK-3β from marking β-catenin for degradation. Since β-catenin is stabilized, it translocates to the nucleus and interacts with T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors to regulate the expression of target genes such as c-Myc, cyclin D1, and MMPs [29,30]. Wnt factors that are known to activate this β-catenin-dependent pathway include Wnt1, Wnt3a, and Wnt7a [31].

The β-catenin-independent pathways primarily regulate cell motility and polarity and include the planar cell polarity (PCP) pathway and the calcium pathway, although more β-catenin-independent pathways are continuously being reported [32]. In the PCP pathway, Fz activates Jun-N-terminal kinase, a MAP kinase. In the calcium pathway, various Wnt and Fz homologs activate calcium/calmodulin-dependent kinase II and protein kinase C [33]. These pathways have been shown to be upregulated in GBMs and are known to be activated by Wnt2, Wnt4, Wnt5a, Wnt5b, Wnt6, and Wnt11 [31,34].

The aberrant activation of the Wnt pathway promotes cancer progression in many cancer types [30]. In GBM, several players of the β-catenin-dependent pathways have been shown to be important for invasion. β-Catenin is overexpressed in gliomas and its knockdown *in vitro* reduced the invasiveness of GBM cells [35]. EGFR activation disrupts the association of α-catenin with β-catenin, allowing transactivation of β-catenin [36]. Additionally, c-MET has also been shown to activate the Wnt/β-catenin pathway in GBM [37]. The Wnt ligands Wnt1 and Wnt3a were found to be significantly overexpressed in tumors derived from grade III gliomas and GBMs. The knockdown of Wnt1 caused formation of smaller intra-cranial tumors in mice that were non-invasive while the knockdown of Wnt3a completely prevented tumor formation [28]. Knockdown of