Dysregulated Signaling Pathways in Glioblastoma Cancer Stem-Like Cells: Potential Targets for Therapeutic Intervention

Abstract
Glioblastoma multiforme (GBM) is the most common and aggressive form of brain cancer. Despite advances in current therapeutic procedures including surgery, chemotherapy and radiation, there have been no significant improvements in patient survival. GBM tumors are highly heterogeneous and it is believed that a small proportion of the tumor mass is comprised of cancer stem-like cells (CSCs). The CSCs behave much like neural stem cells in that they can self-renew and undergo differentiation; however, their high tumor-initiating capacity and therapeutic resistance apparently drive tumorigenesis. Recent evidence depicts the involvement and crosstalk of several different signaling pathways in the regulation and progression of GBM. In this review, we discuss the PI3K-Akt, mTOR, Notch and JAK-STAT signaling pathways that often crosstalk to maintain GBM CSC survival. Furthermore, we discuss the potential role of epigenetic regulation of the CSCs. We believe that a thorough understanding of the signaling pathways that regulate GBM CSCs, and further molecular characterization of the GBM tumors, will lead to the development of more efficient therapies.

Keywords: Glioblastoma; Cancer stem cells; Signaling pathways

Abbreviations: CSCs: Cancer Stem-Like Cells; EGFR: Epidermal Growth Factor Receptor; GBM: Glioblastoma Multiforme; JAK: Janus Tyrosine Kinase; MTOR: Mechanistic Target of Rapamycin; MAPK: Mitogen-Activated Protein Kinase; NF1: Neurofibromin-1; NF-kB: Nuclear Factor-Kappa B; PI3K: Phosphoinositide-3-Kinase; PDGFRA: Platelet Derived Growth Factor Receptor-Alpha; STAT: Signal Transducer and Activator of Transcription

Introduction
Glioblastoma
Glioblastoma multiforme (GBM) is an aggressive and lethal form of brain cancer with an extremely dismal prognosis. Despite advances in the molecular characterization of GBM and new targeted therapeutic approaches, the average patient survival remains only between 12 to 15 months [1]. GBM tumors are highly heterogeneous and are comprised of multiple differentiated cell types and a small subpopulation of cancer stem-like cells (CSCs). The CSCs are believed to be responsible for tumor heterogeneity [2,3], are relatively resistant to treatment, and are responsible for the aggressive nature of the disease [4,5]. Therefore, in order to design new targeted therapeutic approaches it is imperative to identify and characterize the signaling pathways involved in the formation, maintenance and regulation of CSCs. Recent reports on the dedifferentiation of GBM cell lines into more CSC-like cells may have added a new layer of complexity into treating GBM [5,6]. Therefore, in GBM it is critical to target not only the CSCs but also the bulk differentiated cells within the tumor.

Multiple signaling pathways, including Notch1, mechanistic target of rapamycin (mTOR), AKT, mitogen-activated protein kinase (MAPK), Hedgehog and the Janus tyrosine kinase (JAK)- signal transducer and activator of transcription (STAT) pathways, have been found to be involved in the maintenance and progression of GBM [7]. Additionally, mutations of epidermal growth factor receptor (EGFR), platelet derived growth factor receptor-alpha (PDGFRA), phosphatase and tensin homolog (PTEN), p53, and neurofibromin-1 (NF1) are frequently seen in GBM [8]. EGFR is amplified in 40% to 50% of GBM, and gain of function EGFRvIII mutations are found in nearly half of all GBMs [9,10]. The activation of EGFR signaling leads to the subsequent activation of the PI3K-Akt signaling cascade [11], which plays a critical role in cell survival. Despite the identification of EGFR mutations in GBM, the EGFR tyrosine kinase inhibitors, Erlotinib and gefitinib, have only limited clinical benefit [12,13]. Studies indicate that PTEN loss in GBM, which results in the activation of the PI3K-Akt signaling pathway, promotes resistance to EGFR inhibitors [12].

Dysregulating signaling pathways in glioblastoma
Activating mutations of the phosphoinositide-3-kinase (PI3K)-Akt pathway are present in 90% of GBMs [9]. Mutations of the PI3K pathway are often accompanied by PTEN loss, and lead to the hyper activation of the mTORC1 pathway [14]. The mTOR kinase exists as two distinct complexes, mTORC1 and mTORC2 (Figure 1). mTORC1 lies downstream of the PI3K pathway and promotes cell proliferation, protein translation and maintains the energy status of the cancer cell [14,15]. In contrast to mTORC1, the role of mTORC2 is less understood. It was recently found that the combination of the gain of function EGFRvIII mutation and PTEN loss can promote mTORC2 signaling in GBM [16]. Despite
the significance of mTOR signaling in glioma cell growth, only limited success has been achieved from rapamycin (sirolimus), an allosteric mTORC1 inhibitor, in phase I and phase II clinical trials [17]. The limited success of the EGFR and mTORC1 inhibitors indicates that other signaling pathways are involved in GBM and continue to transmit downstream signals thereby promoting cancer cell survival. Furthermore, it is important to define the crosstalk between different pathways that operate in GBM, which can subsequently identify a specific group of targets that can eventually be targeted by therapy.

The Notch signaling cascade is an evolutionarily conserved pathway that regulates cell fate decisions during early embryonic development, and plays important roles in cell differentiation, proliferation, survival, angiogenesis and migration [18-20]. Notch is involved with the maintenance of neural progenitors and the generation of glia during normal brain development [21], and plays critical roles in the maintenance of glioma CSCs (Figure 1) [22]. Recent reports have indicated that Notch inhibitors can induce differentiation of glioma CSCs [23]. Our recent studies indicate that the STAT3 and nuclear factor-kappa B (NF-κB) transcription factors are involved in regulating Notch1 expression and downstream signaling in glioma CSCs (Figure 1) [24]. Furthermore, glioma CSCs were relatively sensitive to STAT3 and NF-κB inhibitors, and the STAT3 inhibitor WP1066 led to marked tumor regression and loss of tumorigenicity in vivo [24,25].

Epigenetic modifications in glioblastoma

While the identification of the multiple pathways operating in GBM provides targets for therapy, recent evidence suggests that epigenetic changes in collaboration with genetic alterations in GBM provide a new layer of complexity into approaching therapeutic intervention [26,27]. The reactivation of the Oct4 and Sox2 transcription factors by DNA methyltransferase promoter transactivation enables GBM cells to develop more stem-like characteristics [28]. Due to the reversibility of epigenetic marks, treatment of GBM CSCs with HDAC inhibitors and demethylating agents, which modulate the conformation of chromatin and regulate gene expression [29], can reactivate silenced tumor suppressor genes [27]. Studies indicate that DNA methyltransferase 5-azacytidine reduced glioma cell proliferation, induced differentiation and reduced tumor growth [30]. Identification of several GBM CSC targets enables the use of combination therapies that may provide maximum benefit. Recent studies indicate that the HDAC inhibitors trichostatin A and valproic acid reduce GBM CSC growth, reduce the expression of stem cell markers and promote cell differentiation [31]. HDAC inhibitors used in combination with the EGFR inhibitor erlotinib has provided promising results in patients with GBM that over express EGFR [32].

Cancer stem-like cells in glioblastoma

Another important point to consider is that the heterogeneity in GBM tumors may reflect that there is more than one population of GBM CSCs, which would provide an additional complication for therapeutic intervention to selectively target the CSC compartment. Previous studies using genomic profiling identified...
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four molecular subtypes of GBM: Proneural, Neural, Classical and Mesenchymal [33]. However, single cell analysis of GBM tumor xenografts has demonstrated that multiple molecular subtypes exist within a single tumor and that individual cells varied markedly in their gene expression patterns [34]. Using traditional tumorsphere culture conditions to enrich for GBM CSCs, as well as growth as adherent CSCs on laminin-coated plates, we have recently shown that both of these CSC populations display stem cell properties and initiate histologically indistinguishable GBM tumors following xenotransplantation [25]. However, these CSC populations differ in their expression patterns when grown in vitro, and induce molecularly distinct tumors in vivo. Taken together, these results suggest that GBM CSCs are not monoclonal but rather are a mosaic of different CSC populations, which are capable of initiating tumors with different gene signatures.

Conclusion

Molecular characterization of GBM over the recent years has enabled the identification of new therapeutic targets. However, the heterogeneous nature of the disease makes it critical to identify multiple pathways that promote cell survival. An important future direction of research is to understand the role of the signaling pathways that are dysregulated in GBM CSCs, and are responsible for the therapeutic resistance and tumor invasiveness of GBM. Moreover, much needs to be learned about the mechanism that underlie the interconversion of bulk GBM cells into GBM CSCs, as well as the different molecular properties of the multiple GBM CSC populations.

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