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Temozolomide resistance and tumor recurrence: Halting the Hedgehog

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Abstract

Chemotherapy with Temozolomide (TMZ), radiation and surgery are the primary methods to treat Glioblastoma Multiforme (GBM), the most common adult intracranial tumor with dismal outcome. GBM resistance to therapy is the main reason of poor patient outcomes. Thus, methods to overcome the resistance are an area of extensive research. This highlight focuses on three recently published articles on the mechanism of resistance and possible therapeutic intervention, including RNA treatment with stem cells. We showed a crucial role of the developmental Sonic Hedgehog (SHH) pathway in the acquisition and maintenance of TMZ resistance. SHH signaling caused TMZ resistance in GBM cells through an increase in the multiple drug resistance gene (MDR1). The SHH receptor, Patched-1 (PTCH1), negatively regulate SHH signaling. In GBM, miR-9 suppressed PTCH1 levels, resulting in the activation of SHH pathway. Thus, SHH signaling is independent of the ligand in resistant GBM cells. MiR-9 was also increased in chemoresistance CD133+ GBM cells. A potential method to reverse resistance was tested by delivering the anti-miR in bone marrow-derived Mesenchymal Stem Cells (MSCs). The anti-miR-9 was transferred into the resistant GBM cells through exosomes and gap junctional intercellular communication. We also review on-going clinical trials with inhibitor of SHH signaling, and also discuss drug delivery by cell therapy for GBM. While GBM treatment has proven to be a challenge, there are a number of novel approaches we are currently developing to manage this malignancy.

Keywords

miR-9; glioblastoma; temozolomide; sonic hedgehog; stem cell; exosome

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Conflicting interests

The author(s) declare that they have no Conflicting interests.

Introduction

Glioblastoma multiforme (GBM) is the most common adult primary tumor of the central nervous system. Clinically, GBM presents a very difficult malignancy to treat, underscored by a 5-year survival of 3–5% [1]. GBM is typically treated with the alkylating agent Temozolomide (TMZ). Since 2010, the monoclonal antibody Bevacizumab (Avastin®) was approved to treat TMZ-resistant patients. GBMs uniformly exhibit resistance to chemotherapy and recur within a few weeks to months [2]. The mechanisms behind this resistance appear to be complex and could occur through synergistic mechanisms, such as cell cycle progression, upregulation of mismatch repair genes and increased activity of ATP-dependent drug efflux pumps [3–5].

Developmental and oncogenic pathways have often been shown to overlap, suggesting “reactivation” of developmental queues in malignancy. Examples of such pathways include those involving Notch, Bone morphogenic proteins, and Sonic Hedgehog (SHH) [6]. Here we review studies involving developmental pathways involving SHH and discuss the regulatory role of miR-9.

Neurodevelopmental of miR-9 regulation of TMZ resistance

MiRNA are small (18–22 bp) non-coding RNA molecules which regulate cellular processes including development, differentiation and oncogenesis [7]. MiRNA regulates protein expression by binding to the 3′ UTR of target mRNA to suppress translation. MiR-9 is a conserved miR known to regulate development, differentiation and migration of cells within the central nervous system [8]. MiR-9 has also been implicated in oncogenesis. In 2011, miR-9 it was shown to be upregulated in GBM cells, and then later shown to be upregulated within a “stem cell-like” subset of GBM cells [9,10]. Recently we reported on a novel target for miR-9 regulation, the SHH receptor, patched-1 (PTCH1) [11]. Interestingly, miR-9 has three genomic loci on chromosomes 1, 5, and 15. In neurodevelopment, miR-9-2 is known to be upregulated, resulting as the main source during development of the brain [12]. Consistent with cell development, our TMZ-resistant GBM model indicated that an upregulation of the miR-9-2 loci was the likely source of mature miR-9.

SHH signaling is essential for the ventral-dorsal patterning of the CNS [13]. Our data revealed a novel method for activation of the SHH signaling pathway. Typical activation occurs by internalization of PTCH1 when bound to SHH. When PTCH1 is internalized, repression of intracellular signaling is released, resulting in the translocation of Gli1 transcription factor [14]. Yet, in our model PTCH1 expression was reduced, but SHH ligand synthesis was unchanged. MiR-9 overexpression in GBM cells resulted in SSH signaling and TMZ resistance. Chemoresistance in GBM cells was determine to be caused by increased expression of the Multiple Drug Resistance (MDR1) gene and ABCG2. Furthermore, patient-derived neurosphere cell lines from recurrent GBM also showed TMZ resistance. In these cell lines, miR-9-2, MDR1, and ABCG2 were also upregulated, while PTCH1 expression was decreased. The studies with the neurospheres from GBM recapitulated the data with cell lines.

Regulating the Sonic Hedgehog Pathway

As part of our recent work we showed a role for Vismodegib (Erivedge®) in combination with TMZ to overcome the sonic hedgehog-dependent resistance. The advantage of Vismodegib therapy is inhibition of Smoothened, downstream of PTCH1, thus circumventing miR-9 activation in SHH signaling [15]. We selected Vismodegib since this drug has been FDA approved for basal cell carcinoma. It has also been shown to be a potent inhibitor of two downstream ABC transporters, ABCG2/BCRP1 and ABCB1/MDR1 [16]. We were able to show increased cell death and TMZ-induced caspase-3 activity, in combination with Vismodegib. The effect of Vismodegib was dose-dependent. A summary of the roles of Vismodegib is highlighted in Figure 1.

Clinical trial NCT00980343 tested the role of Vismodegib in recurrent GBM (prior to or after surgery) for patients where were candidates for surgery. In the pediatric population, Vismodegib is currently being investigated as monotherapy for recurrent pontine glioma patients (NCT01774253). In addition, NCT01601184 is currently a phase I/II study to evaluate the role of Vismodegib along with TMZ for medulloblastoma patients. The validity of such a trial is due to the activation of SHH pathway and PTCH1 inactivating mutations. Together, these trials along with our data show great promise for targeting the SHH pathway with pharmacological inhibitors.

Cancer Stem Cells (CSCs) and Drug Resistance

The CSC hypothesis proposes the existence of self-renewal and tumor repopulating cells in the tumor [17]. The CSCs are believed to be chemoresistant and to have tumor regenerating properties. CD133 cell surface expression has been used as a marker to identify the CSC population within GBMs [18]. We published a number of experiments that showed chemoresistance of GBM CD133+ cells and showed that this was due to the upregulation of miR-9-2 and MDR1 [19].

We first isolated CD133+ cells from U87 and T98G cell lines. Given the “stem cell” nature of CSCs, we asked if delayed cell cycle progression as a possible mechanism of TMZ resistance. Yet, CD133+ cells did not show any difference in cell cycle status when compared to CD133-cells. PTCH1 expression was decreased with concomitant increase in Gli1 expression. Thus we assayed for five possible PTCH1 targeting miRNAs, but only mature miR-9 was found to be increased in CD133+ cells from both cell lines. Specifically, miR-9-2 was upregulated in these cells and transfection with anti-miR-9 resulted in chemosensitivity.

CSC-targeted therapies offer a new opportunity to eradicate the tumor repopulating cells for GBM, which is undoubtedly a fatal malignancy. According to the CSC hypothesis, CSCs are slow cycling cells that provide the fuel for GBM growth and resistance [20]. By eliminating this subset of GBM cells, it would be expected that the tumor progression would be halted with chemotherapy-mediated death of the differentiated and rapidly cycling bulk tumor cells. On the downside for treatment to target CSCs, it would be important not to interfere with endogenous neural stem cells (NSCs).

MiRNA-targeted therapy for GBM

MiRNA-targeted therapy is a recent field with a growing interest in oncology. The current major hurdle in the development of this therapeutic approach is effective tissue targeting [21]. To overcome this difficulty, we recently published research showing the functional transfer of anti-miR-9 from Mesenchymal Stem Cells (MSCs) to GBM cells, through exosomes (Figure 2). MSCs exhibit an innate tropism to GBM cells *in vivo*. A comparison of the source of MSCs, adipose vs. bone marrow, showed no difference in tropism and migration to glioma capabilities [22]. The mechanisms of this tropism towards CNS lesions have been shown to be multifactorial [23]. Recent data has shown that neoadjuvant irradiation of gliomas enhanced MSC migration towards the glioma [24].

We showed that MSCs can communicate with GBM cells through contact-dependent (gap junction formation) and contact-independent (exosome secretion) mechanisms [25]. GBM resistant cells also showed an increase in connexin-43 after treatment with TMZ [26]. Fluorescent-tagged anti-miR-9 was able to transfer most effectively through exosomes and decrease the expression of miR-9 in TMZ-resistant GBM cells. Along with this decrease in miR-9, MDR1 expression was reduced and the GBM cells exhibited greater sensitivity to TMZ.

Currently, the Phase I Clinical Trial NCT02015819 at the City of Hope Medical Center is in the process of assessing the feasibility of Stem Cell-based therapies for recurrent GBM. Although NCT02015819 uses another stem cell, NSCs, the goal is the same as our studies with MSCs; namely to deliver cytosine deaminase in ectopic expression in NSCs [27]. More importantly, this trial will assess the T-cell response to stem cell based therapy.

Conclusions

Overcoming and treating GBM resistance and recurrence has been a difficult clinical challenge. Although medical management of resistance is limited at this time to TMZ and Bevacizumab, we have taken great strides to understand the mechanisms of resistance and thus, attempt personalized and targeted therapy [28]. Our recent data has highlighted the dichotomy known to exist between malignancy and development. The SHH pathway is a well-established pillar of neural development and thus it is no surprise that cancer cells utilize this mechanism to resist therapy and recur. We have shown the potential in using SHH inhibition to enhance TMZ efficacy. In addition, the usage of Vismodegib allowed for simultaneous targeting of SHH and MDR1 [29].

MiRNA, once considered “junk” DNA products, have now become a major area of research, highlighting the role of MiRs in many biological processes. Among the regulatory roles of miRs is the coordination of development and the parallel process, oncogenesis. MiR-9 is known to have critical roles in neuron development and migration [30], yet has been associated with malignancies of the CNS such as GBM and Medulloblastoma [31,32]. MiR-9 also has important roles in the development of cancers outside of the CNS such as breast, colon, cervical, ovarian, and gastric cancer [33–36]. Here we highlighted the oncogenic role

of miR-9 in GBM and provided a mechanism to reverse miR-9 upregulation using exosomal targeted anti-miR-9.

The CSC hypothesis provides an explanation for the heterogeneity and recurrence of tumors such as GBMs. Recent pharmaceutical research aims to target and eradicate CSC populations in GBMs. Here we also highlight our work showing that CSCs exhibit innate resistance to TMZ because of miR-9 upregulation and subsequent MDR1 expression. This opens the door to two novel targets for CSC-based therapy, miR-9 and MDR1.

Current clinical trials indicate the great promise that exists in a future to overcome this uniformly fatal malignancy. GBMs have been shown to activate a number of pathways and utilize various methods of TMZ resistance. Yet, SHH inhibition may prove to be key to overcome the resistance and subsequent recurrence of TMZ-treated GBMs.

Abbreviations

GBM	glioblastoma multiforme
SHH	sonic hedgehog
MSC	mesenchymal stem cell
CSC	cancer stem cell
MDR1	multiple drug resistance gene 1
miR	microRNA
PTCH1	patched-1
NSC	neural stem cell

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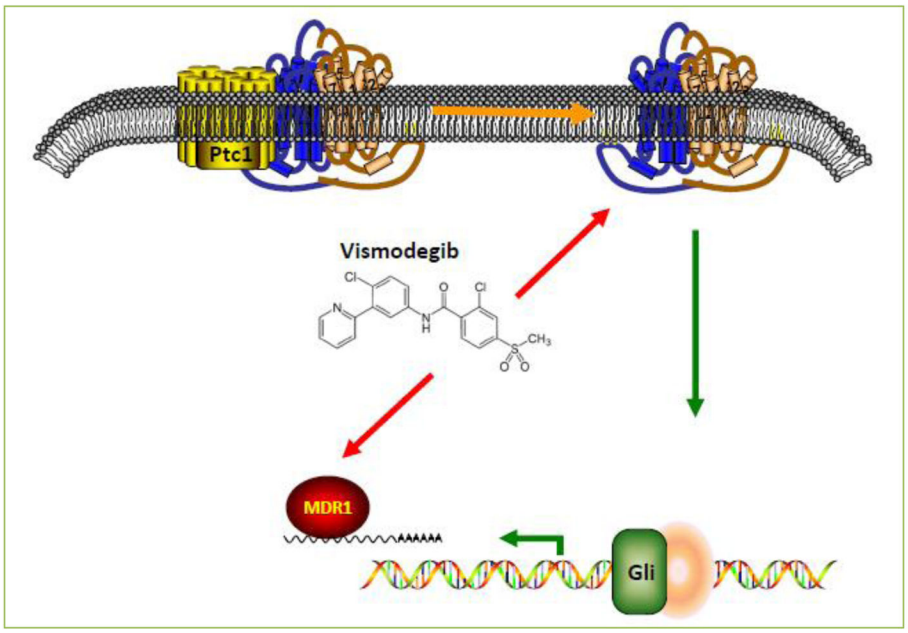

Figure 1. Diagrammatic representation of Vismodegib dual inhibition of SHH Signaling and MDR1 function

Vismodegib inhibits SMO (the SHH activator, yellow) and MDR1 (red) at varying concentrations. This dual inhibition allows for reversal of TMZ resistance in GBM cells and increased TMZ-induced cell death.

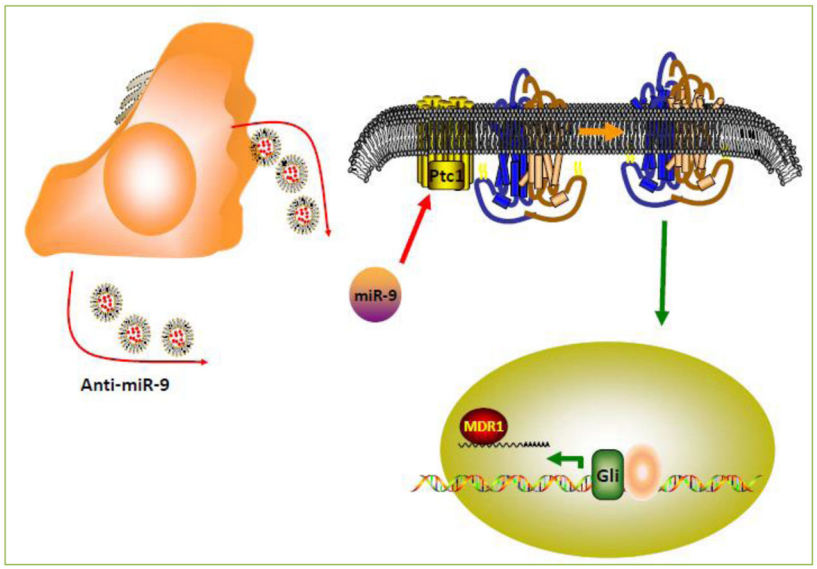

Figure 2. Anti-microRNA-9 targeted therapy by Mesenchymal Stem Cells (MSCs)

MSCs (orange) transfected with fluorescent anti-miR-9 showed intercellular-targeted therapy via MSC-derived Exosomes to GBM cells (yellow). Functional anti-miR-9 decreased endogenous miR-9 and reversed GBM TMZ chemoresistance.