UC Irvine

UC Irvine Previously Published Works

Title

Therapeutic targeting of malignant glioma.

Permalink

<https://escholarship.org/uc/item/9kn9s6c7>

Journal

Anti-cancer agents in medicinal chemistry, 14(8)

ISSN

1871-5206

Authors

Alexandru-Abrams, Daniela
Jadus, Martin R
Hsu, Frank P
et al.

Publication Date

2014

DOI

10.2174/1871520614666140825105145

Peer reviewed

Therapeutic Targeting of Malignant Glioma

Daniela Alexandru-Abrams¹, Martin R. Jatus², Frank P. Hsu¹, Apostolos Stathopoulos^{3,4,5} and Daniela A. Bota^{1,6,7,*}

¹Department of Neurological Surgery, University of California at Irvine; ²Department of Biology, University of California at Irvine; ³Les Cliniques du Sud Luxembourg, Arlon, Belgium; ⁴Department of Neurosurgery C.S.L-Vivalia; ⁵Department of Neurosurgery, University of Sothner California; ⁶Department of Neurology and ⁷The Chao Family Comprehensive Cancer Center, University of California at Irvine

Abstract: Glioblastoma Multiforme (GBM) is the most aggressive primary brain tumor with poor survival rates and universal recurrence despite aggressive treatments. Recent research suggested that GBM has multiple glioma cell populations, some of which are organized in a stem cell hierarchical order with different stages of differentiation. Evidence indicated that recurrence is due to a development or persistence of a subpopulation of these tumor cells which are inherently resistant to treatment and these were defined as the glioma stem-like cells (GSC). It is hypothesized that GSC become highly malignant by accumulating mutations in oncogenic pathways. These cells present with specific surface markers which helps identify them. Targeting the surface markers as well as the signaling pathways of GSCs has been an ongoing research effort. This review focuses on summarizing the current treatment modalities used to glioblastoma treatments, evaluating their efficacy in controlling and eradicating the GSCs, discussing the mechanisms involved in GSC tumor proliferation and resistance to treatments in addition to proposing potential avenues to target GSCs in order to provide a potential cure for this cancer.

Keywords: chemotherapy, glioma stem cells, immunotherapy, malignant glioma, treatment resistance.

INTRODUCTION

Glioblastoma Multiforme (GBM) is the most common primary brain tumor with an incidence of approximately 10,000 new cases annually in the United States [1]. Despite gross total resection, and treatment with radiation and chemotherapy - temozolomide followed by bevacizumab at the time of recurrence -, the median survival has improved only to 18 months in the last decade [1,2] Once the GBMs recur, they are universally fatal, with survival less than a few weeks, despite aggressive treatment [3].

The main cause for GBM recurrence is not precisely known, but recent research suggest that it involves the development (and/or persistence) of subpopulations of tumor cells with resistance to treatment [3]. GBM is characterized by the presence of multiple glioma cell populations [4,5] organized in a stem cell hierarchical order with different stages of differentiation [6]. The cell population situated at the apex of the cellular hierarchy - the glioma stem-like cells (GSC) have been the proposed reason for the recurrence of malignant gliomas after treatment [6-8].

It is hypothesized that glioma cancer stem cells (GSC) can arise either from neural stem or progenitor cells [9] after the accumulation of mutations in oncogenic pathways such as NF1, p53 and PTEN [10] (see Fig. 1). They have the ability to self-renew and to initiate brain tumors [9]. In addition, they express neural stem cell markers and are multipotent [9]. They constitute only a minor subpopulation of the entire tumor. The progeny of GSC are progenitor like cells and differentiated cells, which divide rapidly, and form the bulk of the gliomas [9]. Several surface markers associated with GSC are known [9]. These markers, which include CD133/prominin, Musashi homolog, nestin and A2B5 [3,9], are useful for the isolation and enrichment of GSC.

Previous experiments indicate that "non-stem" cells can produce tumors in orthotopic xenograft models only when implanted in very high numbers [11]. These experimental tumors lack the classical malignant glioma behaviors-namely, an invasive phenotype, vascular

proliferation-, and a limited capacity of tumor initiation [11]. However, orthotopic implantation of a small number of stem-like cells in appropriate animal models generate aggressive growth of tumors [12,13] bringing further support to the stem cell hypothesis for glioma formation [14].

In this review we will first summarize the current treatment modalities used for glioblastoma treatments and evaluate their effectiveness in controlling and eradicating the GSC. We will review the mechanisms involved in GSC-driven tumor proliferation, invasion and resistance to treatment and consider potential therapeutic targets that might hinder or block these oncogenic pathways. Finally, we will discuss potential avenues to target GSC in order to decrease the tumor burden and potentially provide a cure for this type of cancer.

Current Therapeutic Modalities: Glioma Stem Cells Resist the Current, First-Line Treatments for Malignant Gliomas - Radiation and Temozolomide (TMZ)

The standard of care for malignant gliomas after resection is temozolomide (TMZ) plus radiation therapy. A phase III clinical study showed a survival benefit by adding TMZ to postoperative radiation treatment [2,15]. The upregulation of O⁶-methylguanine-DNA methyltransferase (MGMT) expression counteracts the effects of alkylating agents [16], such as TMZ. *In vivo*, the expression of MGMT correlated well with the resistance of malignant gliomas to TMZ treatment [17]. Ultimately, almost all the patients relapsed after treatment with radiation and temozolomide, with a progression free survival of zero at 5 years [18].

The main mechanism through which radiation damages and kills glioma cells is the induction of breakage of the hydrogen bonds within the DNA strands, altering the base pairs, inducing substitutes, destruction of sugars and forming dimers. However, GSC are resistant to radiation [13]. The mechanisms involved in this radio-resistance are the following:

1. Selective cellular growth arrest, or quiescence, is described as the primary means by which the GSC evade the radiation and TMZ chemotherapy induced cellular damage [9,19]. During quiescence, GSC are maintained in growth arrest by various cellular processes and signaling pathways. Once the radiation

*Address correspondence to this author at the University of California, Irvine, Shambrom Hall, Suite 121, Mail Code: 4280, Irvine, CA 92697; Tel: (714) 456-7214; E-mail: dbota@uci.edu

Fig. (1). Glioma Stem Cell Hypothesis: Malignant Transformation of Neural Stem/Progenitor Cells. Under certain conditions such as acquiring a constellation of oncogenic mutations in various pathways (NF1, PTEN or p53), neural stem cells can become glioma stem cells. NF1=Neurofibromin 1, PTEN=Phosphatase and tensin homolog.

and TMZ chemotherapy is completed, the arrested GSC can again become actively proliferating leading to fatal tumor recurrences [7,13,20,21].

2. Activation of checkpoint proteins is a major mechanism for radio-resistance of GBM. The most common checkpoint proteins overexpressed in GSC are ChK1 and ChK2 kinases [13]. These proteins are also expressed in high quantities in tumors isolated from population of cells enriched by radiation, such as CD133+ GSC populations [13]. Early analysis of tumor samples found that patients who had a high percentage of CD133+ GSC had a worse outcome than those patients with a lower percentage of CD133+ GSC [13]. Since the CD133+ GSC population was enriched after radiation suggested activation of DNA repair machinery in these cells. Further investigations showed that CD133+ cells were also able to repair the damage to their DNA more effectively than the CD133- GSC [13]. Treating CD133+ GSC with a specific ChK1 and ChK2 kinase inhibitors sensitized them to radiotherapy, suggesting that one of the main pathways for resistance to radiation therapy was the activation of the DNA damage checkpoint response in GSC [13].

Temozolomide (TMZ) is an alkylating agent that methylates the DNA thereby inhibiting DNA replication and cell proliferation. The GSC are resistant to TMZ, *via* multiple mechanisms of resistance [8].

1. GSC have higher levels of expression of O⁶ – methyl – guanine – DNA – methyltransferase (MGMT) [14,22]. MGMT is a DNA repair protein which reverses the alkylation in the O⁶ position of guanine and thus compensates for the DNA methylation effects [16,22-24]. *In vivo*, the expression of MGMT correlates well with the resistance of malignant gliomas to TMZ treatment [17,23,24]. Bleau *et al* showed that in a mouse model of glioma, treatment with TMZ increases the population of GSC [14]. However, GSC with normal levels of MGMT were resistant to TMZ [25], while only the MGMT negative GSC were killed by TMZ [26]. In the clinical setting, the patients

who had methylation of the MGMT promoter – which leads to silencing of the promoter and prevents gene expression – benefited with the addition of TMZ to their treatment [15]. Chen *et al* showed that a restricted cell population propagates glioblastoma growth after TMZ chemotherapy [8]. A relatively quiescent GCS population was identified after TMZ chemotherapy, that subsequently produced the highly proliferative cells [8].

2. Another mechanism of resistance to TMZ treatments is expression of multiple drug transporting proteins including ABC (ATP binding cassette) transporters which expel out of the cell the chemotherapeutics. Hirsmanmann –Jax *et al* showed that a side population (SP) of cancer stem cells isolated from tumors and identified by flow cytometry contains these transporters and resists cytotoxic drug treatment. They also showed that ABCG2 and ABCA3 transporters were increased in GSC [27]. We showed that GSC have 10 times higher levels of ABCG2 than NSC [3]. Multidrug resistance 1 (MDR1) protein was also over-expressed in TMZ resistant CD133+ GSC [28]. CD133 was initially described as a marker which resisted the uptake of fluorescent markers, which coincidentally resemble chemotherapeutic drugs [22,29].
3. The evasion of the cell-death pathway is another important mechanism of resistance developed by GSC. By losing various proteins and overexpression of other proteins, GSC can modulate their response to chemotherapy and select the most resistant phenotype [9]. One mechanism of avoidance of cell-death pathways is the deletion of phosphatase and tensin homolog (PTEN) in glioma stem cells [14,30]. PTEN suppresses the Akt phosphorylation in this pathway, by reversing the PI3K phosphorylation, thus inhibiting cell proliferation [30]. Therefore, loss of PTEN leads to uncontrolled activation of Akt and tumor growth [14,30]. Bleau *et al* showed treatment with TMZ selects for GSC that lack the PTEN gene [14]. This loss leads to activation of Akt, resistance to apoptosis, tumor progression, and poor prognosis for the patient.

- Several other mechanisms also contribute to the resistance to TMZ treatment. The apoptosis pathway is heavily exploited by GSC [31]. Anti-apoptotic genes, such as Bcl-2, were found to have higher expression in the GSC population which was resistant to TMZ [31]. The addition of XIAP inhibitors increases sensitivity to chemotherapy of the previously chemotherapy resistant GSC [32], thus suggesting that activation of antiapoptotic factors such as XIAPs, are involved in resistance of stem cells to TMZ chemotherapy.
- Constitutive activation of the Notch pathway also increases the oncogenic potential of these cells and maintains their stem cell status [33]. Wang *et al* showed that blocking Notch pathway leads to increased sensitivity of GSC to radiation [34]. By upregulating PI3K/AKT levels and increasing the levels of Bcl-2 family proteins, the Notch pathway produces radioresistance in GSC. The knockdown Notch models increase the sensitivity of GSC to radiation [34].
- Insulin like growth factor binding protein 2 (IGFBP2) can mediate the activation of AKT pathway leading to resistance to radiation and conventional chemotherapy [35]. IGFBP2 upregulates metalloproteinase-2 and CD24, which increases the ability of GSC to invade adjacent tissues [35]. *In vitro* inhibition of IGFBP2 in GSC lead to decreased AKT activation, increased GSC sensitivity to radiation and chemotherapy, as well as decreased stem cell gene expression [36].

Therapeutic Targets that may Hinder or Block GSC-Driven Tumor Proliferation, Invasion and Resistance to Treatment Tumor Angiogenesis and Glioblastoma Stem Cells

GSC induce vascular endothelial growth factor (VEGF) over-expression which increases angiogenesis, leads to new formation of blood vessels to sustain tumor growth and therefore increases blood vessel density [7,9,37] (Fig. 2). Vascularization plays a significant role in the providing nutrition and oxygen for gliomas [9].

Bevacizumab (Avastin) is a humanized monoclonal antibody, that binds and inhibits the activity of VEGF both *in vitro* and *in vivo* [38,39]. Several groups showed that anti-VEGF therapy specifically blocked GSC pro-angiogenic effects [9]. Bevacizumab has been tested in clinical trials for GBM patients, and was approved by the FDA for patients who experienced first or second recurrence of GBM [40]. Nagane *et al.* investigated bevacizumab as single-agent in patients with recurrent malignant gliomas, and found limited benefits in terms of overall survival [41]. Hainsworth *et al* also investigated the benefit of bevacizumab in phase II clinical trial in conjunction with radiation therapy and temozolomide and everolimus as first line treatment of patients with glioblastoma [42]. In this study 61% of treated patients with combination therapy had objective responses [42].

VEGF expression is controlled by the hypoxia induced factors (HIFs). Under hypoxic conditions both GSC and non-stem cells

Fig. (2). Signaling Mechanisms Important in Glioma Stem Cells and Potential Therapeutic Targeting (Part 1). Multiple stem cell and oncogenic pathways are involved in GSC-driven tumor proliferation, invasion and resistance to treatment. The most actively targeted by therapeutic agents are angiogenesis (VEGF inhibition) and differentiation (Notch inhibition). VEGF=Vascular endothelial growth factor, FT=Farnesyltransferase, RAS=Rat sarcoma gene family, MEK=Mitogen-activated protein kinase, ERK=Extracellular regulated kinase, gp60/80=60 kD/ 80 kD glycoprotein, JAK=Janus kinase, STAT3=Signal transducer and activator of transcription 3, BMP=Bone morphogenetic proteins, SMAD=Homolog of both the Drosophila protein, mothers against decapentaplegic (MAD) and the Caenorhabditis elegans protein SMA (small body size), TGFβ=Transforming growth factor beta, Sox=Sry-related HMG box, NICD=Intracellular domain of Notch, cMYC=Myelocytomatosis viral oncogene, Olig2/Olig4=Oligodendrocyte lineage transcription factor 2/4, TRRAP=Transformation/transcription domain-associated protein, NF-κB= nuclear factor kappa-light-chain-enhancer of activated B cells.

gliomas express VEGF, but the expression was higher in the GSC group [43,44]. Li *et al* found the HIF-1 α and HIF-2 α separately controlled VEGF expression in GSC [43]. Hypoxic conditions also select for the GSC fraction of the tumor cells and can also induce expression of the certain stem cell markers [39,45,46].

Hypoxia Inducible Factor HIF-1 α Maintains the Tumorigenic Potential of Gliomas Stem Cells and Increases Treatment Resistance

Hypoxia is common in tumor growth, and for many years it was believed to inhibit tumor growth. However, recent studies indicate that hypoxia actually contributes to tumor growth and proliferation. In malignant gliomas, hypoxia was found to promote angiogenesis, tumor growth and radioresistance [47]. Hypoxic niches play an essential role in the maintenance of GSC [43,44,47]. These "hypoxic niches" were also involved in the maintenance of normal stem cells [9]. In embryonic stem cells, hypoxia maintains the self-renewal potential and prevents the differentiation of neural stem cells. Yoshida *et al* demonstrated that hypoxia enhanced production of induced pluripotent stem cells (iPSC) [48]. GBM frequently display numerous GSC around the areas of necrosis [43]. CD133 is also a marker for hypoxic stress [49]. This may be the reason why some GBM GSC might be selected for in the hypoxic regions.

In addition, hypoxia increases the expression of GSC markers [39,47]. Previous work indicates that hypoxia increases CD133+ GSC [50,51]. In hypoxic conditions, GSC activate HIF-1 α thus increasing their self-renewal ability and anti-differentiated status of GSC [52]. Notch signaling is important in hypoxia, as it maintains the cells in an undifferentiated state. Activation of Notch signaling pathway occurs by recruiting HIF-1 α at Notch responsive promoters [53]. In addition, multiple HIF regulated genes were reported to be expressed in GSC, but not in glioma tumor cells [52]. Studies showed that losing factors such as HIF-2 α for example, leads to a significant decrease in both GSC proliferation and self-renewal in cultures, and decrease in tumorigenic potential in animals [52]. In addition expression of HIF-1 α and HIF2 α was found to lead to an increase in oncogenicity of GSCs [47]. Therefore HIF-1 α family proteins represent a solution for targeting GSC populations. There are a number of HIF1 α targeted agents such as polyamides, quinols and naphthoquinone spiroketal analogues, shikonin derivatives, epidithiodiketopiperazines, and two representative drugs: echinomycin and bortezomib. Only bortezomib was tried in clinical studies of GBM patients with limited results [54].

Signaling Pathways in GSC not only Lead to Resistance to Chemotherapy, but also Induce New Tumor Formation and Lead to Recurrence

Malignant gliomas are highly infiltrative tumors displaying both radiation and conventional chemotherapy resistances. They frequently recur locally despite gross total surgical resection. As described above, cellular quiescence of the gliomas stem cells allows them to evade the TMZ chemotherapy and radiation induced damage. Several signaling pathways, including Notch, Hedgehog and transforming growth factors such as TGF β , are involved in maintaining quiescence in GSC that helps these cells evade systemic treatments (Fig. 3).

Notch Signaling Pathway Increases the Oncogenic Potential of GSCs

The Notch signaling pathway is believed to maintain the cellular quiescence of GSC [55]. This pathway was implicated in the self-renewal potential of the stem cells, inhibiting differentiation and protecting the cells from excessive usage of the proliferative potential [55]. In T cell acute lymphoblastic leukemia and in lymphoma, Notch was implicated in maintaining the cancer stem cells [56]. In gliomas, the Notch pathway activating mutations were associated with purified CD133+ GSC [57]. It is believed that in GSC Notch activation makes the cells quiescent and helps them escape radio-chemotherapy treatment [9]. These cells are then

released from their quiescent dormancy and enter the cell cycle and differentiate into tumors [9]. In glioblastoma sphere cultures treated with gamma secretase inhibitors (GSIs), the CD133+ GSCs were depleted and the putative GSC markers were downregulated (CD133, nestin, BMI1, Olig2) [58]. In these experiments, treatment with GSIs leads to the inhibition of tumor sphere formation *in vitro* and to reduced tumor size in the xenograft model [58]. Further investigations showed that Notch signaling blockade depletes oncogenic GSC through reduced cells proliferation and increased cell apoptosis associated with decreased levels of AKT phosphorylation and STAT3 [33]. Inhibition of the Notch signaling pathway can also target the endothelial cells in malignant gliomas [59]. Hovinga *et al* showed that when the tumor endothelial cells were eliminated from a GBM explant, there was a simultaneous decrease in self-renewal of the tumor stem cells [59]. Combination treatments with Notch blockade and radiation therapy resulted in a decrease in proliferation and self-renewal of the tumor explants [59]. Thus the Notch pathway links angiogenesis and GSC; this allows a dual targeting approach for future treatments. RO4929097 is a gamma secretase inhibitor of Notch signaling which has been used in Phase I trial in patients with advanced solid malignancies with promising results [60] and could have immediate relevance for GBM.

The Hedgehog – GLI is also Involved in Maintaining the Oncogenic Potential of Gliomas Stem Cells

Hedgehog (HH) –GLI signaling pathway regulates self – renewal and tumor-forming abilities in CD133+ GSC [61]. Blockade of the HH pathway using cyclopamine depletes GSC [25]. The homeobox gene, Nanog, was recently identified as a mediator for HH-GLI and contributes to the expansion of the CD133+ GSC population, and maintaining glioblastoma growth [61]. It has also been shown that a loss of p53 contributes to up-regulation of Nanog by activating HH pathway and by negatively regulating the activity and level of GLI [61]. GLI was also found to upregulate Notch and downregulate BMP signaling, which is a pro-differentiation action on stem cells [62]. GANT61 is a preclinical molecule targeting the GLI1 and GLI2 in the HH –GLI pathway causes apoptosis in myeloid leukemia cells [63].

Epidermal Growth Factor Receptor (EGFR) Signaling Pathway is Involved in Maintaining the Oncogenic Potential of Gliomas Stem Cells

Epidermal growth factor receptor (EGFR) signaling pathway is involved in maintenance of GSC with a malignant phenotype [64]. Approximately 40-60 % of GBM tumors exhibit EGFR amplification along with a high EGFR protein expression levels [65]. The EGFR activation initiates the phosphatidylinositol-3-kinases (PI3K)/Akt pathway. PI3K are lipid kinases that phosphorylate lipid phosphatidylinositol (4, 5)-bisphosphate (PIP2) to generate phosphatidylinositol (3, 4, 5)-triphosphate (PIP3) [66,67]. PIP3 recruits AKT to the cell membrane, which enhances cell growth, survival, and proliferation of the cells. EGFR variant III (EGFR vIII) possesses a hierarchical model of expression, which was restricted to epigenetic mechanisms which are characteristic of GSC [68]. Inhibition of the EGFR pathway *via* tyrosine kinase inhibitors induced apoptosis in CD133+ GSCs [69,70], while inhibition of AKT activity lead to suppression of self-renewal in GSC and CD133+ GSC apoptosis [71]. *In vitro* studies showed that it is possible to target only GSC and not NSC, due to lower levels of EGFR expression on the NSC surfaces [3]. Clinical trials targeting the EGFR variant III (EGFR vIII) have had promising results [72,73] in the GBM patient population.

Transforming Growth Factor beta (TGF β) Enhances the Tumor Formation Ability of Glioma Stem Cells

Transforming growth factor beta (TGF β) is a very powerful cytokine demonstrably involved in many cellular processes including embryonal development, cell growth, differentiation,

Fig. (3). Signaling Mechanisms Important in Glioma Stem Cells and Potential Therapeutic Targeting (Part 2). Aberrant Growth Factors Expression, Adhesion Molecules Modulation as well as Protein Degradation and Differentiation Pathways are involved in GSC-driven tumor proliferation, invasion and resistance to treatment. The most actively targeted by therapeutic agents are aberrant growth factor receptor expression (EGF, PDGF, VEGF), adhesion (integrins), protein degradation (proteasome inhibitors) and differentiation (Hedgehog inhibitors). HH= Hedgehog, Ptc1=Patched homolog 1, SMO=Smoothened, Gli1/2/3=Glioma protein 1/2/3, EGF=Epidermal growth factor, PDGF=Platelet-derived growth factor, PI3K= Phosphatidylinositol 3-kinase, PIP2=Plasma membrane aquaporin 2/3, PTEN=Phosphatase and tensin homolog, Akt=Protein Kinase B (PKB), VEGF= Vascular endothelial growth factor, TSC=Tuberous sclerosis complex 1 gene, mTOR=Mammalian target of rapamycin cMYC=Myelocytomatosis viral oncogene, Olig2/Olig4=Oligodendrocyte lineage transcription factor 2/4, TRRAP=Transformation/transcription domain-associated protein, NF- κ B=Nuclear factor kappa-light-chain-enhancer of activated B cells.

morphogenesis, wound healing and immune system regulation [74]. When expressed within GSC, the expression of TGF β is associated with self-renewal and tumorigenic potential of GSC. This occurs by the induction of LIF through Smad complex binding to LIF promoter [75] *via* the activation of JAK-STAT pathway [75]. Therefore decreasing the secretion of TGF β and inhibiting the JAK-STAT pathway can lead to a decrease in self renewal and tumorigenic potential in GSC [75].

TGF β signaling maintains the tumorigenic capacity of GSC *via* induction of SOX2 expression, which was promoted by induction of SOX4 [76]. SOX4 is a direct target gene of TGF β signaling, and SOX4 associates with SOX2 enhancer region, promoting its expression [76]. Silencing SOX2 leads to decrease in oncogenicity and self-renewal in GSC [77]. The undifferentiated phenotype of GSC is one of the key criteria for retaining the tumorigenic potential of these cells [76,77]. Cox *et al* showed that SOX2 overexpression can be correlated with either poor prognosis or increased tumorigenesis [78]. Annovazzi *et al* also showed that SOX2 overexpression was correlated with highest malignancy grade [79]. Alonso *et al* also demonstrated that SOX2 overexpression induced invasion and migration in gliomas stem cells [80]. In addition, knockdown experiments showed that SOX2 was essential for maintaining stem cell phenotype in malignant cells [80].

Platelet Derived Growth Factor Receptor (PDGFR) Augments GSC Tumorigenic Capacity

PDGFR plays an important role in the developing and adult brain. This protein is also over expressed in GBM at the transcriptional level. *In vivo* studies correlated abnormal PDGF signaling with glioma formation [81]. In animal studies, PDGF blocked neuroblast generation and enhanced neural stem cell proliferation in the subventricular zone with formation of glioma like hyperplasia [82]. PDGFR β was recently reported to be highly expressed in GSC [83]. Pharmacological inhibition of PDGFR β decreases GSC self-renewal potential, survival, tumor growth, and invasion [83]. Imatinib, a tyrosine kinase inhibitor, which inhibits the PDGF signaling pathway, reduces the ability of GSC to differentiate [84].

The c-Myc Oncogene Maintains the Tumorigenic Potential in Glioma Stem Cells

The oncogenic transcription factor c-Myc, is involved in activating the expression of many genes through several mechanisms, such as recruitment of histone acetylase, chromatin remodeling factors, and interaction with basal transcription factors [85]. Guney *et al* showed that c-Myc inactivation induces telomere independent senescence [85]. C-Myc is highly expressed in GSC, and c-Myc knockdown reduces cell proliferation, induction of cell apoptosis

and loss of oncogenic potential [45]. In addition, inactivation of p53 and PTEN – tumor suppressor genes – leads to increased expression of c-Myc and increase in oncogenicity of GSC [86]. Inhibitors of c-Myc were found to induce cells cycle arrest and apoptosis in acute myeloid leukemia cells [87]. To date, there are no c-Myc inhibitors used in the treatment of malignant gliomas.

Bmi1 Contributes to the GSC Maintenance and Transformation

Bmi1 is an epigenetic silencer gene [88]. Under normal circumstances it is involved in determination of the differential of stem cell in several tissues [88] and it is a positive regulator of neural stem cells [88]. Bruggeman *et al* demonstrated that Bmi1 was involved in malignant transformation of both neural and differentiated astrocytes [89]. Bmi1 is over-expressed malignant gliomas [89]. To date efforts are on the way to identify small molecule inhibitors to Bmi1.

Over-Expression of Chemokine Receptors Leads to GSC Migration

Over-expression of chemokine receptors such as CXCR4, is a common mechanism related to GSC migration [22]. For glioma cells to migrate, a complex combination of multiple molecular mechanisms is needed, including alteration of tumor cell adhesion molecules, secretion of proteases, modification of actin cytoskeleton, and acquisition of resistance to apoptosis (by affecting PI3K, Akt, mTOR, NF-kappaB regulated pathways), and autophagy (programmed cell death type II) [90]. *In vitro* CXCR4 inhibition synergizes with cytotoxic chemotherapy in gliomas [91]. Inhibiting the migration of GSC contains the tumor and prevents invasion. There are several CXCR4 inhibitors, developed for use specifically in HIV patients, but they have not yet been tested malignant gliomas.

Adhesion Molecules Play an Important Role in Migration and thus are Important in Maintaining the Oncogenic Potential of Glioma Stem Cells

Adhesion molecules play an important role in nervous system development, and in neural migration and differentiation [92]. One such molecule is L1CAM [92], which regulates neuronal cell growth, survival and migration and axonal outgrowth and neurite extension during development. It is expressed in gliomas and other cancers and makes it a good potential cell surface target [19]. The L1CAM knockdown expression in GSC disrupted the sphere formation ability of GSC, suppressed tumor growth while inducing apoptosis [19]. L1CAM is found in association with CD133+ cells, while CD133- cells are L1CAM negative [19]. Studies involving lentiviral *shRNA* targeting of L1CAM disrupted neurosphere formation induced apoptosis and inhibited growth of CD133+ GSC [19]. Currently there are no drugs targeting specifically the L1CAM molecule. However, based on *in vitro* results, this is seems to be an attractive target for glioma growth inhibition.

Integrins, another class of adhesion molecules, are cell surface receptors that are expressed during development and mediate development specific events by binding matrix ligands [93]. Integrin 6 α was shown to be important in the neural migration during olfactory development [93]. Recently it was shown that integrin 6 α is highly expressed in GSC [94] and that by directly interacting with laminin on endothelial cells increases the oncogenicity of GSC. Disruption of the interaction and targeting of integrin 6 α inhibits self-renewal, and proliferation and tumor formation potential [94]. Cilengitide is a specific inhibitor of α v β 3 and α v β 5 integrins and it was tested in phase II clinical trials with promising activity against glioma [95].

Bone Morphogenic Protein (BMP) Increased Expression Leads to Increased Stem Cell Phenotype in Malignant Gliomas

BMP plays an important role in determining the cell fate during development of the nervous system. Piccirillo *et al* showed that BMP can activate receptors in GSCs to induce their differentiation

in xenograft models [96]. By direct implantation of BMP beads into malignant gliomas, the researchers obtained slower tumor growth in xenograft models suggesting that this could be a new therapy in the treatment of malignant gliomas [96]. Another group of researchers showed that GSC are capable of regulating and alter the BMP receptors in order to induce a more malignant phenotype for GSCs [97]. Based on this evidence, BMP could be used as a very promising treatment for malignant gliomas.

CD133 is used for the Identification and Enrichment of GSC Populations and is also a Potential Target for Therapy

CD133 is a cell surface protein found on the surface of a population of GSC. CD133+ GSC are tumorigenic and have proliferative activity [98], and that the presence of CD133+ cells is an independent risk factor for tumor recurrence and inversely correlates with patient survival in patients with malignant gliomas [99]. Liu *et al* found that purified CD133+ GSC express a series of genes which are associated with undifferentiated, slow-growing, migrating and an anti-inflammatory and anti-angiogenic phenotype [57]. Multiple studies have shown that CD133+ glioma cells are resistant to chemotherapy [50,100] potentially because CD133+ GSC express higher levels of proteins associated with chemotherapy resistance, such as the DNA repair protein MGMT [14] and the drug transporter gene ABCG2/BCRP (breast cancer resistance protein) [27]. CD133+ cells also have high mRNA levels of other apoptosis inhibitors, including FLIP, Bcl-2, Bcl-X and some IAP family genes [22].

The role of CD133 marker in targeting GSC holds great potential. However, the task of developing a drug against malignant gliomas targeting this marker remains difficult as there are GSC populations which do not express the marker on their surface [101,102]. Beier *et al* describe CD133- GSCs, and postulate that this molecular heterogeneity of GSC may contribute to the molecular heterogeneity of GBM [102]. In addition, Beier *et al*, demonstrated that CD133+ GSC from high grade astrocytomas can give rise to CD133- progenitor cells [101]. These cells however, had significant telomere shortening when compared to the CD133+ compartment [101]. Beier *et al* also isolated CD133+ GSC and CD133- GSC populations from different GBM [3]. Both cell subtypes showed similar tumor inducing abilities in nude mice [103]. However, clinically the CD133- GBM subtypes showed lower proliferative index [103]. The researchers also showed by GeneArray analysis of these samples that the 133+ and 133- GSC had as much as 117 genes that were differentially expressed [103].

Overcoming GSC Immune Surveillance Escape-Immunomodulation Therapy in the Treatment of Glioblastoma Multiforme

Earlier studies in gliomas showed that CD133+ cells did not express detectable Major Histocompatibility Complex (MHC) class I or natural killer (NK) cell activating ligands [98], thus these cells were resistant to adaptive and innate immune surveillance. Incubating GSC with interferon gamma significantly increased the percentage of CD133+ cells that expressed MHC class I and natural killer ligands [98]. In addition, when the CD133+ cells were pretreated with interferon gamma, they became sensitive to NK cell-mediated lysis *in vitro* [98]. GSC can be attacked using active immunotherapy by designing vaccines that stimulate the host's intrinsic immune response to the tumor. The initial immunotherapies against gliomas included irradiated whole tumor cell inoculation engineered to secrete cytokines [104], or combined with cytokine secreting cells [105] or cytokines alone [106]. TR2-01849 is a specific antibody to CD133 protein, specifically designed to target glioblastoma stem cells. Various immune strategies, such as adjuvants, heat shock proteins, γ δ T cell treatments have been used as immunomodulators of the immune system in the treatment of GBM [107].

A newer method of triggering the active immunity is the development of a dendritic cell (DC) vaccine, which used patient-derived professional antigen presenting cells (APC), such as

dendritic cells, to initiate the tumor-specific T-cell response when re-injected in the patients [108-112]. Using this procedure, the lysates of GSC produced a more robust immune system response than using lysates of GBM cells. The DC conditioned with GBM GCS lysates present a wide variety of antigens to T cells to stimulate effective anti-tumor immunity and it is believed that DC vaccination strategies using GSC lysates generate more effective stronger immune responses against a series of more specific epitopes. It has been known that in monoclonal gammopathy patients can develop an immune response against specific epitopes such as SOX2 [113]. In the case of gliomas, Pellegatta *et al* showed that the use of GSC lysates elicited a strong T cell immune response [114]. In addition, DC vaccination using the antigens/lysates derived from the “mesenchymal subtype” of GBM (which has a very poor prognosis when compared to the pro-neural GBM [97,115]) produced a better survival than those other types of GBM treated with their respective lysate-pulsed DC; i.e., classical or pro-neural types [116].

miRNA can Modulate Tumor Cell Proliferation, Invasion, Apoptosis and Senescence

The miRNA are small, non -coding RNAs, which down-regulate gene expression post transcriptionally during different cell processes such as apoptosis, differentiation and development [117]. miRNA were originally identified as potential tumor suppressor molecules which induces apoptosis in neuroblastoma cells [118]. The miRNA expression in gliomas is associated with GSC maintenance and growth [117,119]. For gliomas, miR-34a was down-regulated when compared to normal brain [117,119]. Mutant p53 expressing glioma tumors had lower levels of miR-34a than wildtype p53 tumors. The presence of miR-34a within those gliomas inhibits their proliferation pathways, cell survival, migration and invasion [117,119]. Transfection of cells with miR-34a inhibited oncogene expression such as c-Met, Notch-1 and Notch-2 [117,119]. miR-34a expression induces gliomas stem cells differentiation and thus has the potential to be used in targeting the oncogenic pathways. Another group identified that miR-204 suppressed self – renewal, stem cell phenotype and migration ability of glioma cells [120]. The mechanism was consistent with targeting the transcriptional factor SOX4 and the migration-promoting receptor EphB2 [120]. MiR-204 expression decreased invasiveness and increased host survival [120]. It appeared that the miR-204 is an important regulator of the development of glioma stem cell phenotype in regular malignant gliomas [120]. Currently there are no clinical trials which use the miRNA concept. However, as technology advances, it is very likely that miRNA could be used as a treatment strategy.

LOW GRADE GLIOMAS AND GLIOMA STEM CELLS

Some publications attempted to investigate the relationship between low grade gliomas and glioma stem cells, as GBM frequently develop from low grade gliomas. Thon *et al* demonstrated that CD133+ cells isolate from gliomas of different grades had the same properties concerning self-renewal and multi-lineage differentiation [121]. Rebetz *et al.* analyzed the surface marker expression of stem cells (CD133), glial progenitors (PDGFRalpha, A2B5, O4, and CD44); and late oligodendrocyte progenitors (O1) [122]. The data suggested that low grade gliomas cells expressed glial progenitor markers [122]. The oligodendrocyte progenitor was weakly expressed on all low grade gliomas and the majority of high-grade gliomas [122]. This study indicated that low grade gliomas have the potential for glial lineage progenitor cell expansion, and therefore have the potential of becoming malignant.

DISCUSSION AND CONCLUSIONS

A significant challenge in designing treatment against GSC is to avoid damage to the neural stem cells and progenitor cells, which share many genetic similarity and antigen expression profiles with GCS [7]. The adult human stem cells serve important functions in tissue repair after injury, especially after traumatic brain injury,

ischemic and tumor associated or treatment induced destruction [9]. These normal neural stem cells (NSC) also possess some intrinsic activity against tumor cells, they are attracted by glioma cells *in vivo* and they induce apoptosis and inhibit glioma stem cell growth [123]. The neural stem cells and the progenitor cells are, however, more sensitive than GSC to temozolomide and carboplatin chemotherapy [3]. Hypoxic conditions and EGFR expression seem to be most characteristic to GSC and not to NSC. We previously showed that bortezomib, a proteasome inhibitor, and erlotinib, an EGF tyrosine kinase inhibitor, decrease the viability of GSC, but not to affect NSC [3].

New research also shows the need to target both the GSC and the bulk of the glioma cells. In their new paper, Chen *et al* indicated that if one targets only the GSC, the mice still die due to their tumor burden [8]. Therefore, there is a need for more effective drugs or immunotherapeutic strategies that target both GSC and the more differentiated glioma cell mass. More clinical trials focused on combination therapies against multiple oncogenic pathways involved in GSC maintenance, division and differentiation are still needed.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

- [1] Yamada, R.; Nakano, I. Glioma stem cells, their role in chemoresistance. *World Neurosurg.*, **2012**, 77(2), 237-240.
- [2] Stupp, R.; Mason, W.P.; Van den Bent, M.J.; Weller, M.; Fisher, B.; Taphoorn, M.J.; Belanger, K.; Brandes, A.A.; Marosi, C.; Bogdahn, U.; Curschmann, J.; Janzer, R.C.; Ludwin, S.K.; Gorlia, T.; Allgeier, A.; Lacombe, D.; Cairncross, J.G.; Eisenhauer, E.; Mirimanoff, R.O. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.*, **2005**, 352(10), 987-996.
- [3] Gong, X.; Schwartz, P.H.; Linskey, M.E.; Bota, D.A. Neural stem/progenitors and glioma stem-like cells have differential sensitivity to chemotherapy. *Neurology*, **2011**, 76(13), 1126-1134.
- [4] Glas, M.; Rath, B.H.; Simon, M.; Reinartz, R.; Schramme, A.; Trageser, D.; Eisenreich, R.; Leinhaas, A.; Keller, M.; Schildhaus, H.U.; Garbe, S.; Steinfarz, B.; Pietsch, T.; Steindler, D.A.; Schramm, J.; Herrlinger, U.; Brüstle, O.; Scheffler, B. Residual tumor cells are unique cellular targets in glioblastoma. *Ann. Neurol.*, **2010**, 68(2), 264-269.
- [5] Wick, W.; Wick, A.; Weiler, M.; Weller, M. Patterns of progression in malignant glioma following anti-VEGF therapy, perceptions and evidence. *Curr. Neurol. Neurosci. Rep.*, **2011**, 11(3), 305-312.
- [6] Chen, R.; Nishimura, M.C.; Bumbaca, S.M.; Kharbanda, S.; Forrest, W.F.; Kasman, I.M.; Greve, J.M.; Soriano, R.H.; Gilmour, L.L.; Rivers, C.S.; Modrusan, Z.; Nacu, S.; Guerrero, S.; Edgar, K.A.; Wallin, J.J.; Lamszus, K.; Westphal, M.; Heim, S.; James, C.D.; VandenBerg, S.R.; Costello, J.F.; Moorefield, S.; Cowdrey, C.J.; Prados, M.; Phillips, H.S. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. *Cancer Cell*, **2010**, 17(4), 362-375.
- [7] Bao, S.; Wu, Q.; Sathornsumetee, S.; Hao, Y.; Li, Z.; Hjelmeland, A.B.; Shi, Q.; McLendon R.E.; Bigner, D.D.; Rich, J.N. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res.*, **2006**, 66(16), 7843-7848.
- [8] Chen, J.; Li, Y.; Yu, T.S.; McKay, R.M.; Burns, D.K.; Kernie, S.G.; Parada, L.F. A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature*, **2012**, 488(7412), 522-526.
- [9] Huang, Z.; Cheng, L.; Guryanova, O.A.; Wu, Q.; Bao, S. Cancer stem cells in glioblastoma-molecular signaling and therapeutic targeting. *Protein & Cell*, **2010**, 1(7), 638-655.
- [10] Alcantara Llaguno, S.; Chen, J.; Kwon, C.H.; Jackson, E.L.; Li, Y.; Burns, D.K.; Alvarez-Buylla, A.; Parada L.F. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer Cell*, **2009**, 15(1), 45-56.
- [11] Singh, S.K.; Hawkins, C.; Clarke, I.D.; Squire, J.A.; Bayani, J.; Hide, T.; Henkelman, R.M.; Cusimano, M.D.; Dirks, P.B. Identification of human brain tumour initiating cells. *Nature*, **2004**, 429(7015), 396-401.

- [12] Hemmati, H.D.; Nakano, I.; Lazareff, J.A.; Masterman-Smith, M.; Geschwind, D.H.; Bronner-Fraser, M.; Kornblum, H.I. Cancerous stem cells can arise from pediatric brain tumors. *Proc. Nat. Acad. Sci. USA*, **2003**, *100*(25), 15178-15183.
- [13] Bao, S.; Wu, Q.; McLendon, R.E.; Hao, Y.; Shi, Q.; Hjelmeland, A.B.; Dewhirst, M.W.; Bigner, D.D.; Rich, J.N. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*, **2006**, *444*(7120), 756-760.
- [14] Bleau, A.M.; Hambardzumyan, D.; Ozawa, T.; Fomchenko, E.I.; Huse, J.T.; Brennan, C.W.; Holland, E.C. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell*, **2009**, *4*(3), 226-235.
- [15] Stupp, R.; Hegi, M.E.; Mason, W.P.; Van den Bent, M.J.; Taphoorn, M.J.; Janzer, R.C.; Ludwin, S.K.; Allgeier, A.; Fisher, B.; Belanger, K.; Hau, P.; Brandes, A.A.; Gijtenbeek, J.; Marosi, C.; Vecht, C.J.; Mokhtari, K.; Wesseling, P.; Villa, S.; Eisenhauer, E.; Gorlia, T.; Weller, M.; Lacombe, D.; Cairncross, J.G.; Mirimanoff, R.O. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study, 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.*, **2009**, *10*(5), 459-466.
- [16] Fruehauf, J.P.; Brem, H.; Brem, S.; Sloan, A.; Barger, G.; Huang, W.; Parker, R. *In vitro* drug response and molecular markers associated with drug resistance in malignant gliomas. *Clin. Cancer Res.*, **2006**, *12*(15), 4523-4532.
- [17] Hermisson, M.; Klumpp, A.; Wick, W.; Wischhusen, J.; Nagel, G.; Roos, W.; Kaina, B.; Weller, M. O6-methylguanine DNA methyltransferase and p53 status predict temozolomide sensitivity in human malignant glioma cells. *J. Neurochem.*, **2006**, *96*(3), 766-776.
- [18] Sanai, N.; Berger, M.S. Glioma extent of resection and its impact on patient outcome. *Neurosurgery*, **2008**, *62*(4), 264-266.
- [19] Bao, S.; Wu, Q.; Li, Z.; Sathornsumetee, S.; Wang, H.; McLendon, R.E.; Hjelmeland, A.B.; Rich, J.N. Targeting cancer stem cells through L1CAM suppresses glioma growth. *Cancer Res.*, **2008**, *68*(15), 6043-6048.
- [20] Cheng, L.; Wu, Q.; Guryanova, O.A.; Huang, Z.; Huang, Q.; Rich, J.N.; Bao, S. Elevated invasive potential of glioblastoma stem cells. *Biochem. Biophys. Res. Commun.*, **2011**, *406*(4), 643-648.
- [21] Dietrich, J.; Imitola, J.; Kesari, S. Mechanisms of disease, the role of stem cells in the biology and treatment of gliomas. *Nat. Clin. Pract. Oncol.*, **2008**, *5*(7), 393-404.
- [22] Liu, G.; Yuan, X.; Zeng, Z.; Tunici, P.; Ng, H.; Abdulkadir, I.R.; Lu, L.; Irvin, D.; Black, K.L.; Yu, J.S. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol. Cancer*, **2006**, *5*, 67.
- [23] Kitange, G.J.; Carlson, B.L.; Mladek, A.C.; Decker, P.A.; Schroeder, M.A.; Wu, W.; Grogan, P.T.; Giannini, C.; Ballman, K.V.; Buckner, J.C.; James, C.D.; Sarkaria, J.N. Evaluation of MGMT promoter methylation status and correlation with temozolomide response in orthotopic glioblastoma xenograft model. *J. Neuro-Oncol.*, **2009**, *92*(1), 23-31.
- [24] Kitange, G.J.; Carlson, B.L.; Schroeder, M.A.; Grogan, P.T.; Lamont, J.D.; Decker, P.A.; Wu, W.; James, C.D.; Sarkaria, J.N. Induction of MGMT expression is associated with temozolomide resistance in glioblastoma xenografts. *Neuro-Oncology*, **2009**, *11*(3), 281-291.
- [25] Clement, V.; Sanchez, P.; De Tribolet, N.; Radovanovic, I.; Ruiz i Altaba, A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr. Biol.*, **2007**, *17*(2), 165-172.
- [26] Beier, D.; Röhrig, S.; Pillai, D.R.; Schwarz, S.; Kunz-Schughart, L.A.; Leukel, P.; Proescholdt, M.; Brawanski, A.; Bogdahn, U.; Trampe-Kieslich, A.; Giebel, B.; Wischhusen, J.; Reifenberger, G.; Hau, P.; Beier, C.P. Temozolomide preferentially depletes cancer stem cells in glioblastoma. *Cancer Res.*, **2008**, *68*(14), 5706-5715.
- [27] Hirschmann-Jax, C.; Foster, A.E.; Wulf, G.G.; Nuchtern, J.G.; Jax, T.W.; Gobel, U.; Goodell, M.A.; Brenner, M.K. A distinct "side population" of cells with high drug efflux capacity in human tumor cells. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*(39), 14228-14233.
- [28] Nakai, E.; Park, K.; Yawata, T.; Chihara, T.; Kumazawa, A.; Nakabayashi, H.; Shimizu, K. Enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. *Cancer Invest.*, **2009**, *27*(9), 901-908.
- [29] Farrar EMHaWL. Purification and characterization of cancer stem cells. In: Farrar WL, Ed. *Cancer Stem Cells*, Cambridge University Press; **2009**.
- [30] Martelli, A.M.; Evangelisti, C.; Chiarini, F.; McCubrey, J.A. The phosphatidylinositol 3-kinase/Akt/mTOR signaling network as a therapeutic target in acute myelogenous leukemia patients. *Oncotarget*, **2010**, *1*(2), 89-103.
- [31] Hsieh, A.; Ellsworth, R.; Hsieh, D. Hedgehog/GLI1 regulates IGF dependent malignant behaviors in glioma stem cells. *J. Cell. Physiol.*, **2011**, *226*(4), 1118-1127.
- [32] Vellanki, S.H.K.; Grabrucker, A.; Liebau, S.; Proepper, C.; Eramo, A.; Braun, V.; Boeckers, T.; Debatin, K.-M.; Fulda, S. Small-molecule XIAP inhibitors enhance gamma-irradiation-induced apoptosis in glioblastoma. *Neoplasia*, **2009**, *11*(8), 743-752.
- [33] Fan, X.; Khaki, L.; Zhu, T.S.; Soules, M.E.; Talsma, C.E.; Gul, N.; Koh, C.; Zhang, J.; Li, Y.M.; Maciarczyk, J.; Nikkha, G.; Dimeco, F.; Piccirillo, S.; Vescovi, A.L.; Eberhart, C.G. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells*, **2010**, *28*(1), 5-16.
- [34] Wang, J.; Wakeman, T.P.; Lathia, J.D.; Hjelmeland A.B.; Wang, X.-F.; White, R.R.; Rich, J.N.; Sullenger, B.A. Notch promotes radioresistance of glioma stem cells. *Stem Cells*, **2010**, *28*(1), 17-28.
- [35] Lin, Y.; Jiang, T.; Zhou, K.; Xu, L.; Chen, B.; Li, G.; Qiu, X.; Jiang, T.; Zhang, W.; Song, S.W. Plasma IGFBP-2 levels predict clinical outcomes of patients with high-grade gliomas. *Neuro. Oncology*, **2009**, *11*(5), 468-476.
- [36] Hsieh, D.; Hsieh, A.; Stea, B.; Ellsworth, R. IGFBP2 promotes glioma tumor stem cell expansion and survival. *Biochem. Bioph. Res. Co.*, **2010**, *397*(2), 367-372.
- [37] Folkens, C.; Shaked, Y.; Man, S.; Tang, T.; Lee, C.R.; Zhu, Z.; Hoffman, R.M.; Kerbel, R.S. Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor 1. *Cancer Res.*, **2009**, *69*(18), 7243-7251.
- [38] Vredenburgh, J.J.; Desjardins, A.; Herndon, J.E. 2nd, Marcello, J.; Reardon, D.A.; Quinn, J.A.; Rich, J.N.; Sathornsumetee, S.; Gururangan, S.; Sampson, J.; Wagner, M.; Bailey, L.; Bigner, D.D.; Friedman, A.H.; Friedman, H.S. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *J. Clin. Oncol.*, **2007**, *25*(30), 4722-4729.
- [39] Vredenburgh, J.J.; Desjardins, A.; Herndon, J.E. 2nd, Dowell, J.M.; Reardon, D.A.; Quinn, J.A.; Rich, J.N.; Sathornsumetee, S.; Gururangan, S.; Wagner, M.; Bigner, D.D.; Friedman, A.H.; Friedman, H.S. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. *Clin. Cancer Res.*, **2007**, *13*(4), 1253-1259.
- [40] Möller, S.; Grunnet, K.; Hansen, S.; Schultz, H.; Holmberg, M.; Sorensen, M.; Poulsen, H.S.; Lassen, U. A phase II trial with bevacizumab and irinotecan for patients with primary brain tumors and progression after standard therapy. *Acta Oncol.*, **2012**, *51*(6), 797-804.
- [41] Nagane, M.; Nishikawa, R.; Narita, Y.; Kobayashi, H.; Takano, S.; Shinoura, N.; Aoki, T.; Sugiyama, K.; Kuratsu, J.; Muragaki, Y.; Sawamura, Y.; Matsutani, M. Phase II study of single-agent bevacizumab in Japanese patients with recurrent malignant glioma. *Jpn. J. Clin. Oncol.*, **2012**, *42*(10), 887-895.
- [42] Hainsworth, J.D.; Shih, K.C.; Shepard, G.C.; Tillinghast, G.W.; Brinker, B.T.; Spigel, D.R. Phase II study of concurrent radiation therapy, temozolomide, and bevacizumab followed by bevacizumab/everolimus as first-line treatment for patients with glioblastoma. *Clin. Adv. Hematol. Oncol.*, **2012**, *10*(4), 240-216.
- [43] Li, Z.; Bao, S.; Wu, Q.; Wang, H.; Eyler, C.; Sathornsumetee, S.; Shi, Q.; Cao, Y.; Lathia, J.; McLendon, R.E.; Hjelmeland, A.B.; Rich, J.N. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell*, **2009**, *15*(6), 501-513.
- [44] Heddleston, J.M.; Li, Z.; Lathia, J.D.; Bao, S.; Hjelmeland, A.B.; Rich, J.N. Hypoxia inducible factors in cancer stem cells. *Br. J. Cancer*, **2010**, *102*(5), 789-795.
- [45] Wang, J.; Wang, H.; Li, Z.; Wu, Q.; Lathia, J.D.; McLendon, R.E.; Hjelmeland, A.B.; Rich, J.N. c-Myc is required for maintenance of glioma cancer stem cells. *PLoS One*, **2008**, *3*(11), e3769.
- [46] Saidi, A.; Javerzat, S.; Bellahcene, A.; De Vos, J.; Bello, L.; Castronovo, V.; Deprez, M.; Loiseau, H.; Bikfalvi, A.; Hagedorn, M. Experimental anti-angiogenesis causes upregulation of genes associated with poor survival in glioblastoma. *Int. J. Cancer*, **2008**, *122*(10), 2187-2198.
- [47] Heddleston, J.M.; Li, Z.; McLendon, R.E.; Hjelmeland, A.B.; Rich, J.N. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle*, **2009**, *8*(20), 3274-3284.
- [48] Yoshida, Y.; Takahashi, K.; Okita, K.; Ichisaka, T.; Yamanaka, S. Hypoxia enhances the generation of induced pluripotent stem cells. *Cell Stem Cell*, **2009**, *5*(3), 237-241.

- [49] Griguer, C.E.; Oliva, C.R.; Gobin, E.; Marcotelles, P.; Benos, D.J.; Lancaster, J.R. Jr.; Gillespie, G.Y. CD133 is a marker of bioenergetic stress in human glioma. *PLoS One*, **2008**, 3(11), e3655.
- [50] Blazek, E.R.; Foutch, J.L.; Maki, G. Daoy medulloblastoma cells that express CD133 are radioresistant relative to CD133- cells, and the CD133+ sector is enlarged by hypoxia. *Int. J. Radiat. Oncol. Biol. Phys.*, **2007**, 67(1), 1-5.
- [51] Bar, E.E.; Lin, A.; Mahairaki, V.; Matsui, W.; Eberhart, C.G. Hypoxia increases the expression of stem-cell markers and promotes clonogenicity in glioblastoma neurospheres. *Am. J. Pathol.*, **2010**, 177(3), 1491-1502.
- [52] Soeda, A.; Park, M.; Lee, D.; Mintz, A.; Androutsellis-Theotokis, A.; McKay, R.D.; Engh, J.; Iwama, T.; Kunisada, T.; Kassam, A.B.; Pollack, I.F.; Park, D.M. Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1 α . *Oncogene*, **2009**, 28(45), 3949-3959.
- [53] Gustafsson, M.V.; Zheng, X.; Pereira, T.; Gradin, K.; Jin, S.; Lundkvist, J.; Ruas, J.L.; Poellinger, L.; Lendahl, U.; Bondesson, M. Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev. Cell*, **2005**, 9(5), 617-628.
- [54] Friday, B.B.; Anderson, S.K.; Buckner, J.; Yu, C.; Giannini, C.; Geoffroy, F.; Schwerkoske, J.; Mazurek, M.; Gross, H.; Pajon, E.; Jaekle, K.; Galanis, E. Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma, a north central cancer treatment group study. *Neuro-oncology*, **2012**, 14(2), 215-221.
- [55] Chapouton, P.; Skupien, P.; Hesl, B.; Coolen, M.; Moore, J.C.; Madelaine, R.; Kremmer, E.; Faus-Kessler, T.; Blader, P.; Lawson, N.D.; Bally-Cuif, L. Notch activity levels control the balance between quiescence and recruitment of adult neural stem cells. *J. Neurosci.*, **2010**, 30(23), 7961-7974.
- [56] Jundt, F.; Schwarzer, R.; Dorken, B. Notch signaling in leukemias and lymphomas. *Curr. Mol. Med.*, **2008**, 8(1), 51-59.
- [57] Liu, Q.; Nguyen, D.H.; Dong, Q.; Shitaku, P.; Chung, K.; Liu, O.Y.; Tso, J.L.; Liu, J.Y.; Konkankit, V.; Cloughesy, T.F.; Mischel, P.S.; Lane, T.F.; Liao, L.M.; Nelson, S.F.; Tso, C.L. Molecular properties of CD133+ glioblastoma stem cells derived from treatment-refractory recurrent brain tumors. *J. Neurooncol.*, **2009**, 94(1), 1-19.
- [58] Anthony, T.E.; Mason, H.A.; Gridley, T.; Fishell, G.; Heintz, N. Brain lipid-binding protein is a direct target of Notch signaling in radial glial cells. *Genes Dev.*, **2005**, 19(9), 1028-1033.
- [59] Hovinga, K.E.; Shimizu, F.; Wang, R.; Panagiotakos, G.; Van Der Heijden, M.; Moayedpardazi, H.; Correia, A.S.; Soulet, R.; Major, T.; Menon, J.; Tabar, V. Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. *Stem Cells*, **2010**, 28(6), 1019-1029.
- [60] Tolcher, A.W.; Messersmith, W.A.; Mikulski, S.M.; Papadopoulos, K.P.; Kwak, E.L.; Gibbon, D.G.; Patnaik, A.; Falchook, G.S.; Dasari, A.; Shapiro, G.I.; Boylan, J.F.; Xu, Z.X.; Wang, K.; Koehler, A.; Song, J.; Middleton, S.A.; Deutsch, J.; Demario, M.; Kurzrock, R.; Wheeler, J.J. Phase I study of rO4929097, a gamma secretase inhibitor of notch signaling, in patients with refractory metastatic or locally advanced solid tumors. *J. Clin. Oncol.*, **2012**, 30(19), 2348-2353.
- [61] Zbinden, M.; Duquet, A.; Lorente-Trigos, A.; Ngwaby, S.N.; Borges, I.; Ruiz i Altaba, A. NANOG regulates glioma stem cells and is essential *in vivo* acting in a cross-functional network with GLI1 and p53. *EMBO J.*, **2010**, 29(15), 2659-2674.
- [62] Stecca, B.; Ruiz i Altaba, A. A GLI1-p53 inhibitory loop controls neural stem cell and tumour cell numbers. *EMBO J.*, **2009**, 28(6), 663-676.
- [63] Pan, D.; Li, Y.; Li, Z.; Wang, Y.; Wang, P.; Liang, Y. Gli inhibitor GANT61 causes apoptosis in myeloid leukemia cells and acts in synergy with rapamycin. *Leuk. Res.*, **2012**, 36(6), 742-754.
- [64] Mazzoleni, S.; Politi, L.S.; Pala, M.; Cominelli, M.; Franzin, A.; Sergi, S.; Falini, A.; De Palma, M.; Bulfone, A.; Poliani, P.L.; Galli, R. Epidermal growth factor receptor expression identifies functionally and molecularly distinct tumor-initiating cells in human glioblastoma multiforme and is required for gliomagenesis. *Cancer Res.*, **2010**, 70(19), 7500-7513.
- [65] Frederick, L.; Wang, X.Y.; Eley, G.; James, C.D. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer Res.*, **2000**, 60(5), 1383-1387.
- [66] Hatiboglu, M.A.; Wei, J.; Wu, A.S.; Heimberger, A.B. Immune therapeutic targeting of glioma cancer stem cells. *Target Oncol.*, **2010**, 5(3), 217-227.
- [67] Cheng, C.K.; Fan, Q.W.; Weiss, W.A. PI3K signaling in glioma-animal models and therapeutic challenges. *Brain Pathol.*, **2009**, 19(1), 112-120.
- [68] Del Vecchio, C.A.; Giacomini, C.P.; Vogel, H.; Jensen, K.C.; Florio, T.; Merlo, A.; Pollack, J.R.; Wong, A.J. EGFRvIII gene rearrangement is an early event in glioblastoma tumorigenesis and expression defines a hierarchy modulated by epigenetic mechanisms. *Oncogene*, **2013**, 32(21), 2670-2681.
- [69] Soeda, A.; Inagaki, A.; Oka, N.; Ikegame, Y.; Aoki, H.; Yoshimura, S.; Nakashima, S.; Kunisada, T.; Iwama, T. Epidermal growth factor plays a crucial role in mitogenic regulation of human brain tumor stem cells. *J. Bio. Chem.*, **2008**, 283(16), 10958-10966.
- [70] Griffero, F.; Daga, A.; Marubbi, D.; Capra, M.C.; Melotti, A.; Pattarozzi, A.; Gatti, M.; Bajetto, A.; Porcile, C.; Barbieri, F.; Favoni, R.E.; Lo Casto, M.; Zona, G.; Spaziante, R.; Florio, T.; Corte, G. Different response of human glioma tumor-initiating cells to epidermal growth factor receptor kinase inhibitors. *J. Biol. Chem.*, **2009**, 284(11), 7138-7148.
- [71] Eyler, C.E.; Foo, W.C.; LaFiura, K.M.; McLendon, R.E.; Hjelmeland, A.B.; Rich, J.N. Brain cancer stem cells display preferential sensitivity to Akt inhibition. *Stem Cells*, **2008**, 12, 3027-3036.
- [72] Heimberger, A.B.; Archer, G.E.; Crotty, L.E.; McLendon, R.E.; Friedman, A.H.; Friedman, H.S.; Bigner, D.D.; Sampson, J.H. Dendritic cells pulsed with a tumor-specific peptide induce long-lasting immunity and are effective against murine intracerebral melanoma. *Neurosurgery*, **2002**, 158(164), discussion 64-66.
- [73] Sampson, J.H.; Archer, G.E.; Mitchell, D.A.; Heimberger, A.B.; Bigner, D.D. Tumor-specific immunotherapy targeting the EGFRvIII mutation in patients with malignant glioma. *Semin. Immunol.*, **2008**, 20(5), 267-275.
- [74] Aigner, L.; Bogdahn, U. TGF-beta in neural stem cells and in tumors of the central nervous system. *Cell Tissue Res.*, **2008**, 331(1), 225-241.
- [75] Penuelas, S.; Anido, J.; Prieto-Sanchez, R.M.; Folch, G.; Barba, I.; Cuatrecasas, I.; Garcia-Dorado, D.; Poca, M.A.; Sahuquillo, J.; Baselga, J.; Seoane, J. TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell*, **2009**, 15(4), 315-327.
- [76] Ikushima, H.; Todo, T.; Ino, Y.; Takahashi, M.; Miyazawa, K.; Miyazono, K. Autocrine TGF-beta signaling maintains tumorigenicity of glioma-initiating cells through Sry-related HMG-box factors. *Cell Stem Cell*, **2009**, 5(5), 504-514.
- [77] Gangemi, R.M.; Griffero, F.; Marubbi, D.; Perera, M.; Capra, M.C.; Malatesta, P.; Ravetti, G.L.; Zona, G.L.; Daga, A.; Corte, G. SOX2 silencing in glioblastoma tumor-initiating cells causes stop of proliferation and loss of tumorigenicity. *Stem Cells*, **2009**, 27(1), 40-48.
- [78] Cox, J.L.; Wilder, P.J.; Desler, M.; Rizzino, A. Elevating SOX2 levels deleteriously affects the growth of medulloblastoma and glioblastoma cells. *PLoS one*, **2012**, 7(8), e44087.
- [79] Annovazzi, L.; Mellai, M.; Caldera, V.; Valente, G.; Schiffer, D. SOX2 expression and amplification in gliomas and glioma cell lines. *Cancer Genomics Proteomics*, **2011** 8(3), 139-147.
- [80] Alonso, M.M.; Diez-Valle, R.; Manterola, L.; Rubio, A.; Liu, D.; Cortes-Santiago, N.; Urquiza, L.; Jauregi, P.; Lopez De Munain, A.; Sampron, N.; Aramburu, A.; Tejada-Solis, S.; Vicente, C.; Otero, M.D.; Bandrés, E.; Garcia-Foncillas, J.; Idoate, M.A.; Lang, F.F.; Fueyo, J.; Gomez-Manzano, C. Genetic and epigenetic modifications of Sox2 contribute to the invasive phenotype of malignant gliomas. *PLoS one*, **2011**, 6(11), e26740.
- [81] Calzolari, F.; Malatesta, P. Recent insights into PDGF-induced gliomagenesis. *Brain Pathol.*, **2010**, (3), 527-538.
- [82] Jackson, E.L.; Garcia-Verdugo, J.M.; Gil-Perotin, S.; Roy, M.; Quinones-Hinojosa, A.; VandenBerg, S.; Alvarez-Buylla, A. PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron*, **2006**, 51(2), 187-199.
- [83] Kim, Y.; Kim, E.; Wu, Q.; Guryanova, O.; Hitomi, M.; Lathia, J.D.; Serwanski, D.; Sloan, A.E.; Weilm, R.J.; Lee, J.; Nishiyama, A.; Bao, S.; Hjelmeland, A.B.; Rich, J.N. Platelet-derived growth factor receptors differentially inform intertumoral and intratumoral heterogeneity. *Genes Dev.*, **2012**, 26(11), 1247-62.
- [84] Dong, Y.; Han, Q.; Zou, Y.; Deng, Z.; Lu, X.; Wang, X.; Zhang, W.; Jin, H.; Su, J.; Jiang, T.; Ren, H. Long-term exposure to imatinib reduced cancer stem cell ability through induction of cell differentiation via activation of MAPK signaling in glioblastoma cells. *Mol. Cell Biochem.*, **2012**, 370(1-2), 89-102.
- [85] Guney, I.; Wu, S.; Sedivy, J.M. Reduced c-Myc signaling triggers telomere-independent senescence by regulating Bmi-1 and p16(INK4a). *Proc. Nat. Acad. Sci. USA*, **2010**, 107(10), 3645-3650.
- [86] Zheng, H.; Ying, H.; Yan, H.; Kimmelman, A.C.; Hiller, D.J.; Chen, A.J.; Perry, S.R.; Tonon, G.; Chu, G.C.; Ding, Z.; Stommel, J.M.; Dunn, K.L.; Wiedemeyer, R.; You, M.J.; Brennan, C.; Wang, Y.A.; Ligon, K.L.; Wong, W.H.; Chin, L.; DePinho, R.A. p53 and

- Pten control neural and glioma stem/progenitor cell renewal and differentiation. *Nature*, **2008** 455(7216), 1129-1133.
- [87] Huang, M.J.; Cheng, Y.C.; Liu, C.R.; Lin, S.; Liu, H.E. A small-molecule c-Myc inhibitor, 10058-F4, induces cell-cycle arrest, apoptosis, and myeloid differentiation of human acute myeloid leukemia. *Exp. Hematology*, **2006**, 34(11), 1480-1489.
- [88] Molofsky, A.V.; Pardoll, R.; Iwashita, T.; Park, I.K.; Clarke, M.F.; Morrison, S.J. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature*, **2003**, 425(6961), 962-967.
- [89] Bruggeman, S.W.; Hulsman, D.; Tanger, E.; Buckle, T.; Blom, M.; Zevenhoven, J.; Van Tellinghen, O.; Van Lohuizen, M. Bmi-1 controls tumor development in an Ink4a/Arf-independent manner in a mouse model for glioma. *Cancer Cell*, **2007** 12(4), 328-341.
- [90] Lefranc, F.; Brothi, J.; Kiss, R. Possible future issues in the treatment of glioblastomas, special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. *J. Clin. Oncol.*, **2005**, 23(10), 2411-2422.
- [91] Redjal, N.; Chan, J.A.; Segal, R.A.; Kung, A.L. CXCR4 inhibition synergizes with cytotoxic chemotherapy in gliomas. *Clin. Cancer Res.*, **2006**, 12(22), 6765-6771.
- [92] Schafer, M.K.; Altevogt, P.; L1CAM malfunction in the nervous system and human carcinomas. *Cell Mol. Life Sci.*, **2010**, 67(14), 2425-2437.
- [93] Whitley, M.; Treloar, H.; De, Arcangelis, A.; Georges, Labouesse, E.; Greer, C.A.; The alpha6 integrin subunit in the developing mouse olfactory bulb. *J. Neurocytol.*, **2005** 34(1-2), 81-96.
- [94] Lathia, J.D.; Gallagher, J.; Heddleston, J.M.; Wang, J.; Eyler, C.E.; Macsworlds, J.; Wu, Q.; Vasanji, A.; McLendon, R.E.; Hjelmeland, A.B.; Rich, J.N. Integrin alpha 6 regulates glioblastoma stem cells. *Cell stem cell*, **6**(5), 421-432.
- [95] Gilbert, M.R.; Kuhn, J.; Lamborn, K.R.; Lieberman F, Wen, P.Y.; Mehta, M.; Cloughesy, T.; Lassman, A.B.; Deangelis, L.M.; Chang, S.; Prados, M. Cilengitide in patients with recurrent glioblastoma, the results of NABTC 03-02, a phase II trial with measures of treatment delivery. *J. Neurooncol.*, **2012** 106(1), 147-53.
- [96] Piccirillo, S.G.; Vescovi, A.L.; Bone morphogenetic proteins regulate tumorigenicity in human glioblastoma stem cells. Ernst Schering Foundation symposium proceedings. **2006**, 5, 59-81.
- [97] Lee, Y.; Scheck, A.C.; Cloughesy, T.F.; Lai, A.; Dong, J.; Farooqi, H.K.; Liao, L.M.; Horvath, S.; Mischel, P.S.; Nelson S.F. Gene expression analysis of glioblastomas identifies the major molecular basis for the prognostic benefit of younger age. *BMC Med. Genom.*, **2008**, 1, 52.
- [98] Wu, A.; Wiesner, S.; Xiao, J.; Ericson, K.; Chen, W.; Hall, W.A.; Low, W.C.; Ohlfest, J.R. Expression of MHC I and NK ligands on human CD133+ glioma cells, possible targets of immunotherapy. *J. Neurooncol.*, **2007**, 83(2), 121-131.
- [99] Zeppernick, F.; Ahmadi, R.; Campos, B.; Dictus, C.; Helmke, B.M.; Becker, N.; Lichter, P.; Unterberg, A.; Radlwimmer, B.; Herold-Mende, C.C. Stem cell marker CD133 affects clinical outcome in glioma patients. *Clin. Cancer Res.*, **2008**, 14(1), 123-129.
- [100] Eramo, A.; Ricci-Vitiani, L.; Zeuner, A.; Pallini, R.; Lotti, F.; Sette, G.; Pilozi, E.; Larocca, L.M.; Peschle, C.; De Maria, R. Chemotherapy resistance of glioblastoma stem cells. *Cell Death Differ.*, **2006**, 13(7), 1238-1241.
- [101] Beier, F.; Beier, C.P.; Aschenbrenner, I.; Hildebrandt, G.C.; Brummendorf, T.H.; Beier, D. Identification of CD133(-)/telomerase (low) progenitor cells in glioblastoma-derived cancer stem cell lines. *Cell Molecular Neurobiol.*, **2011**, 31(3), 337-343.
- [102] Beier, C.P.; Beier, D.; CD133 negative cancer stem cells in glioblastoma. *Front Biosci.*, **2011**, 3, 701-710.
- [103] Beier, D.; Hau, P.; Proescholdt, M.; Lohmeier, A.; Wischhusen, J.; Oefner, P.J.; Aigner, L.; Brawanski, A.; Bogdahn, U.; Beier, C.P. CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res.*, **2007** 67(9), 4010-4015.
- [104] Herrlinger, U.; Kramm, C.M.; Johnston, K.M.; Louis, D.N.; Finkelstein, D.; Reznikoff, G.; Dranoff, G.; Breakefield, X.O.; Yu, J.S. Vaccination for experimental gliomas using GM-CSF-transduced glioma cells. *Cancer Gene Ther.*, **1997**, 4(6), 345-352.
- [105] Plautz, G.E.; Miller, D.W.; Barnett, G.H.; Stevens, G.H.; Maffett, S.; Kim, J.; Cohen, P.A.; Shu, S. T. Cell adoptive immunotherapy of newly diagnosed gliomas. *Clin. Cancer Res.*, **2000**, 6(6), 2209-2218.
- [106] Sobol, R.E.; Fakhrai, H.; Shawler, D.; Gjerset, R.; Dorigo, O.; Carson, C.; Khaleghi, T.; Koziol, J.; Shiftan, T.A.; Royston, I. Interleukin-2 gene therapy in a patient with glioblastoma. *Gene Ther.*, **1995** 2(2), 164-167.
- [107] Ge, L.; Hoa, N.; Bota, D.A.; Natividad, J.; Howat, A.; Jadus, M.R.; Immunotherapy of brain cancers, the past, the present, and future directions. *Clin. Dev. Immunol.*, **2010**, 296453.
- [108] Ashley, D.M.; Faiola, B.; Nair, S.; Hale, L.P.; Bigner, D.D.; Gilboa, E.; Bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA induce antitumor immunity against central nervous system tumors. *J. Exp. Med.*, **1997**, 186(7), 1177-1182.
- [109] Yamanaka, R.; Zullo, S.A.; Tanaka, R.; Blaese, M.; Xanthopoulos K.G. Enhancement of antitumor immune response in glioma models in mice by genetically modified dendritic cells pulsed with Semliki forest virus-mediated complementary DNA. *J. Neurosurg.*, **2001**, 94(3), 474-481.
- [110] Soling, A.; Rainov, N.G.; Dendritic cell therapy of primary brain tumors. *Mol. Med.*, **2001**, 7(10), 659-667.
- [111] Ruzevick, J.; Jackson, C.; Phallen, J.; Lim, M. Clinical trials with immunotherapy for high-grade glioma. *Neurosurg. Clin. N. Am.*, **2012**, 23(3), 459-4S70.
- [112] Yamanaka, R.; Kajiwar, K. Dendritic cell vaccines. *Adv. Exp. Med. Biol.*, **2012**, 746, 187-200.
- [113] Spisek, R.; Kukreja, A.; Chen, L.C.; Matthews, P.; Mazumder, A.; Vesole, D.; Jagannath, S.; Zebroski, H.A.; Simpson A.J.; Ritter, G.; Durie, B.; Crowley, J.; Shaughnessy, J.D. Jr.; Scanlan, M.J.; Gure, A.O.; Barlogie, B.; Dhodapkar, M.V. Frequent and specific immunity to the embryonal stem cell-associated antigen SOX2 in patients with monoclonal gammopathy. *J. Exp. Med.*, **2007**, 204(4), 831-840.
- [114] Pellegatta, S.; Poliani, P.L.; Corno, D.; Menghi, F.; Ghielmetti, F.; Suarez-Merino, B.; Caldera, V.; Nava, S.; Ravanini, M.; Facchetti, F.; Bruzzone, M.G.; Finocchiaro, G. Neurospheres enriched in cancer stem-like cells are highly effective in eliciting a dendritic cell-mediated immune response against malignant gliomas. *Cancer Res.*, **2006**, 66(21), 10247-1052.
- [115] Phillips, H.S.; Kharbanda, S.; Chen, R.; Forrest, W.F.; Soriano, R.H.; Wu, T.D.; Misra, A.; Nigro, J.M.; Colman, H.; Soroceanu, L.; Williams, P.M.; Modrusan, Z.; Feuerstein, B.G.; Aldape, K. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*, **2006**, 9(3), 157-173.
- [116] Prins, R.M.; Soto, H.; Konkankit, V.; Odesa, S.K.; Eskin, A.; Yong, W.H.; Nelson, S.F.; Liao, L.M. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. *Clin. Cancer Res.*, **2011**, 17(6), 1603-1615.
- [117] Guessous, F.; Zhang, Y.; Kofman, A.; Catania, A.; Li, Y.; Schiff, D.; Purow, B.; Abounader, R. microRNA-34a is tumor suppressive in brain tumors and glioma stem cells. *Cell Cycle*, **2010**, 9(6), 1031-1036.
- [118] Welch, C.; Chen, Y.; Stallings, R.L. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene*, **2007**, 26(34), 5017-5022.
- [119] Kefas, B.; Comeau, L.; Floyd, D.H.; Selevestov, O.; Godlewski, J.; Schmittgen, T.; Jiang, J.; DiPierro, C.G.; Li, Y.; Chiocca, E.A.; Lee, J.; Fine, H.; Abounader, R.; Lawler, S.; Purow, B. The neuronal microRNA miR-326 acts in a feedback loop with notch and has therapeutic potential against brain tumors. *J. Neurosci.*, **2009**, 29(48), 15161-1518.
- [120] Ying, Z.; Li, Y.; Wu, J.; Zhu, X.; Yang, Y.; Tian, H.; Li, W.; Hu, B.; Cheng, S.Y.; Li, M. Loss of miR-204 expression enhances glioma migration and stem cell like phenotype. *Cancer Res.*, **2013**, 73(2), 990-999.
- [121] Thon, N.; Damianoff, K.; Hegermann, J.; Grau, S.; Krebs, B.; Schnell, O.; Tonn, J.C.; Goldbrunner, R. Presence of pluripotent CD133+ cells correlates with malignancy of gliomas. *Mol. Cell Neurosci.*, **2010**, 43(1), 51-59.
- [122] Rebetz, J.; Tian, D.; Persson, A.; Widegren, B.; Salford, L.G.; Englund, E.; Gisselsson, D.; Fan, X. Glial progenitor-like phenotype in low-grade glioma and enhanced CD133-expression and neuronal lineage differentiation potential in high-grade glioma. *PLoS One*, **2008**, 3(4), e1936.
- [123] Glass, R.; Synowitz, M.; Kronenberg, G.; Walzlein, J.H.; Markovic, D.S.; Wang, L.P.; Gast, D.; Kiwit, J.; Kempermann, G.; Kettenmann, H. Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. *J. Neurosci.*, **2005**, 25(10), 2637-2646.