Review

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RNA Interference-Based Immunomodulation for Glioblastoma Immune Microenvironment: A Review

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Glioblastoma is the most common and devastating primary brain tumor in adults. The clinical treatments of glioblastoma are generally focus on surgical excision, chemotherapy and radiotherapy, while the prognosis remains grim. With the adequate understanding of the unique immunological microenvironment in the central nervous system (CNS) and CNS tumors, immune microenvironment modulation becomes a promising treatment modality in glioma. Studies using RNA interference (RNAi) technology to modulate the immune microenvironment are under an active investigation. Using those therapeutic strategies directly or indirectly react with tumor cells, antigen processing cells (APCs, including microglia and dendritic cells (DCs)) and immune cells (such as T cells and natural killer cells (NK cells)) has been extensively studied. Here, we review the current advances in immune microenvironment modulation for glioblastoma with RNAi approach. We also investigate how the factors, associated with immunosuppression, to combat with glioblastoma, and discuss the deficiency for this anti-glioblastoma strategy. From these, we expect to provide a guidance for the future development of RNAi-based immunomodulation strategy for glioblastoma and establish optimized antitumor therapy for clinical treatments.

KEYWORDS: *RNAi-Based Immune Modulation, The Immunosuppressive Tumor Microenvironment, Glioblastoma, Immunity Responses, RNA Therapeutics.*

CONTENTS

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INTRODUCTION

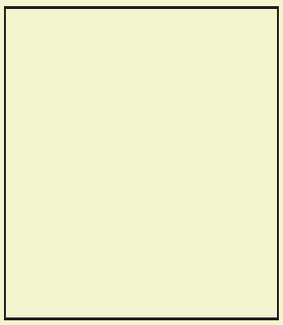
Glioblastoma, one of the most common and malignant primary tumor that originate from glial cells within the central nervous system (CNS), are among the most fatal cancers in human.[1–4] The characteristics of glioblastoma are cellular heterogeneity, fast and extensive infiltration.[5–9] The median survival time for patients diagnosed with the tumors in this category is only 12–18 months.[10–13] The current therapy regimen consists of maximal surgical resection combined with chemotherapy and/or radiotherapy, and they have limited effects on brain tumor progression, recurrence rate or clinical outcome.[14–23] A complex mechanism is involved in the development of glioblastoma, which includes the rapid proliferation of tumor cells along with their ability to evade the immune response mainly due to tumor infiltration of regulatory T cells ($CD4^+CD25^+Foxp3^+$, Tregs).[24–27]

The quite limited efficiency of therapeutics is caused by two major reason:

(i) the disability of most chemotherapeutic agents to cross the blood-brain barrier (BBB),[28–32] and

(ii) the suppressive immune microenvironment.[33–35]

On the one hand, the biological barrier BBB consists of a huge impermeable cellular barrier of capillary endothelium cells connected by tight junctions.[36–39] It significantly protects the CNS from the penetration of toxins and unknown substances from blood and maintain brain homeostasis.[40–42] While the presence of BBB is a great challenge for therapeutic molecules to overcome in order to reach the target site.[43–48] As a consequence, low pharmacological concentrations of therapeutic agents in target



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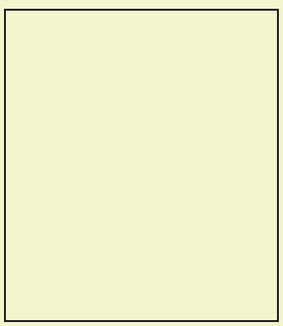

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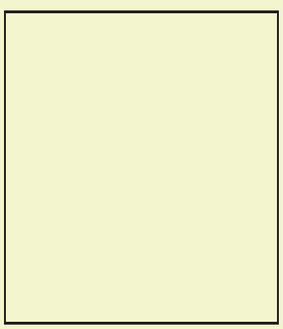

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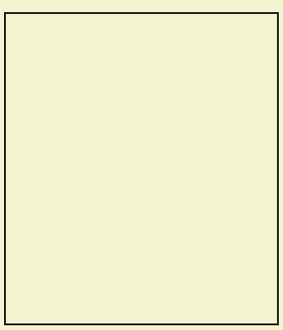

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site within brain tumor induce an insufficient inhibition of tumor progression.[47,49] On the other hand, glioma cells secrete various cytokines, chemokines and growth factors that modulate the local infiltration of various immune cells and the proliferation of T regulatory cells.[50–54]

Although a variety of strategy to treat glioblastoma have been investigated in last decades, the median survival rate of patients remains at 14.6 months after diagnosis.[55,56] The characteristics of glioma are rapid growth, highly infiltrative in nature, and suppressive immune microenvironment, which make glioblastoma particularly difficult to treat.[57–62] Those specific characters motivate brain tumors to establish a self-promoting and mutually self-reinforcing tumor microenvironment (TME) that promotes tumor progression.[63–65] Herein, innovative and efficient therapeutic strategies against the devastating and rapidly fatal disease are urgently needed.

Development of RNA interference (RNAi) technology has opened up new perspectives in human disease treatment, especially glioblastoma.[66–72] The strategy was based on an endogenous pathway that enabled regulation of gene expression, which was identified by Craig Mello and Andrew Fire in 1998.[73,74] In particular, the specific genes of interested were diced from longer RNA transcripts by the RNase-III-like enzyme Dicer, and then assembled into a complex called the RNA-induced silencing complex (RISC), which could direct RNA cleavage, mediate translational repression or induce chromatin modifications (Fig. 1). Hence, researches using RNAi technology to treat glioblastoma as a promising approach have been reported recently.

Tumor microenvironment (TME) is the formidable challenge in advancing the treatment of glioblastoma.[53,75–77] Indeed, the tumor microenvironment is a complicated system, which consists various distinct cell types, multiple signaling pathways, cytokines and extracellular matrix components (EMC).[78–81] The matrix components of TME interact with tumor cells, such as neoplastic cells interact with fibroblasts, vascular endothelial cells, a variety of infiltrating immune cells (including a network of cytokines and chemokines released by these cells), thus promote tumor formation, growth and

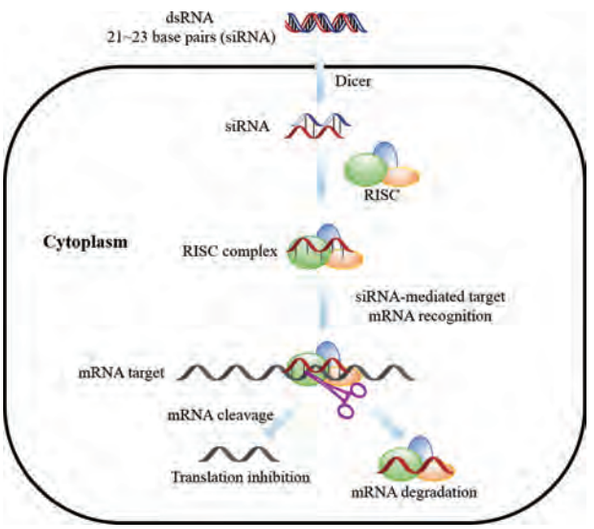

Figure 1. **The mechanism of RNAi process within cell.**

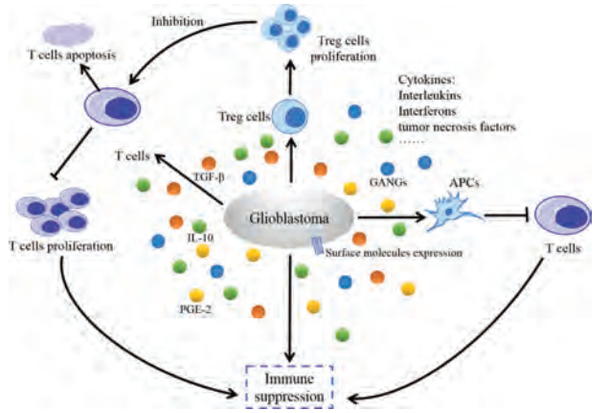

Figure 2. Model of immunosuppressive microenvironment in brain tumors.

metastasis.[64, 81–86] Besides, numerous cytokines, such as interleukins,[87–89] interferons,[90, 91] tumor necrosis factors and so on,[92–95] influence the progression of malignant glioma (Fig. 2). Also, those cytokines have an effect on the immunologic response against gliomas, displaying pro-inflammatory or immunosuppressive activities.[96] The dynamic interplay among those aforementioned factors are intricately coordinates to form an immunosuppressive microenvironment.[97–99] Clarifying the details of immunosuppressive environment of glioma requires consideration not only the intrinsic properties of tumor cells, but also how they interact with those distinct cell types, including antigen processing cells, dendritic cells, microglia, T cells and other cells. Recently, studies using used RNAi to modulate the immunosuppressive microenvironment are now widely investigated. Here, in this review, we will focus on the application of RNAi technology in modulation of immune microenvironment of glioblastoma, clarifying the mechanism of immunosuppression toward different cells, especially between glioma themselves and various immune cells. The recent combinatorial approaches are also discussed from these description. From this review, we expect to provide a future direction of therapeutics of brain tumors for clinical application.

RNAi TECHNOLOGY AND ITS APPLICATION IN BRAIN TUMOR IMMUNE REGULATIONS

A growing number of researches have demonstrated the potential of RNAi technology to provide immune regulations in glioblastoma treatment. With a deeper understanding of the process of development and progression as well as basic biology of the devastating cancer, two primary impediments for efficient treatment are listed: the immunosuppressive effects derived from

(i) glioblastoma (the aberrant secreted factors and the surface immunosuppressive molecules)[100–103] and

(ii) immune associated cell lines (including glioma-associated microglia/macrophages,[104–107] antigen presenting cells, leukocytes, natural killer cells[108–110]).

Each of them will be discussed as followings.

RNAi Toward Glioblastoma

The aberrant expression factors that induce immunosuppressive effects are of great importance in glioblastoma therapeutics.[53, 111–113] A large number of researches have demonstrated that immunosuppressive factors (such as transforming growth factor-β (TGF-β),[114, 115] gangliosides (GANGs),[116–120] interleukin-10 (IL-10)[121–123] and prostaglandin E2 (PGE-2)[124–127] and so on,[128–132]) are actively expressed by glioma. Those soluble factors have a sophisticated and multiple effect on immunosuppressive microenvironment.

TGF-β, for instance, is a multifunctional polypeptide cytokine, which is considered to be one of the major factors responsible for glioma tumorigenesis and TME regulation.[133–135] The dysregulation of TGF-β is involved with various processes, such as fast cell growth, cell survival, differentiation, invasion into normal brain parenchyma and immunosuppressive activity (immune dysfunction).[136–139] RNA interference targeting TGF-β to decrease expression of the activating immune receptor of immune cells and inhibit glioma cells migration and invasiveness as well as abrogate tumorigenicity *in vivo* was studied by Weller and colleagues. Up-regulation of NKG2D, an immune receptor in $CD8^+$ T and NK cells, through a novel therapeutic approach to silence TGF-β gene expression, made glioma cells more efficiently recognized by innate immune recognition via induced self-danger signals disinhibited transcription.[140] In 2013, Hau and colleagues reported that modulated the lactate production by transfecting HTZ-349 glioma cells with siLDH-A (siRNA for lactate dehydrogenase-A) could suppress the level of TGF-β2, which might modulate the migration of glioma cells (Fig. 3).[141] Besides, the immune suppressive component gangliosides could affect lymphocyte responsiveness as well as APCs and T cell functions.[142] Biswas and colleagues reported that GBM-derived gangliosides could induce apoptosis of T cells through involvement of the TGF receptor and activation of the caspase cascade.[120]

Treatment toward glioblastoma by using RNAi technology could induce apoptosis and regulate the aberrant expression factors, thus improving the immunosuppressive microenvironment. However, there are two aspects need to consider for further clinical application. On the one hand, the efficiency of the treatment is not enough for further application due to several reasons, including lack of specific gene targets, the poor selectivity and low efficiency of gene silencing. On the other hand, the immune responses elicited by the host suppress the effector T cell, eliminate the antitumor immunity. Herein, not only the glioma cells, but the other cells in TME should be take into account

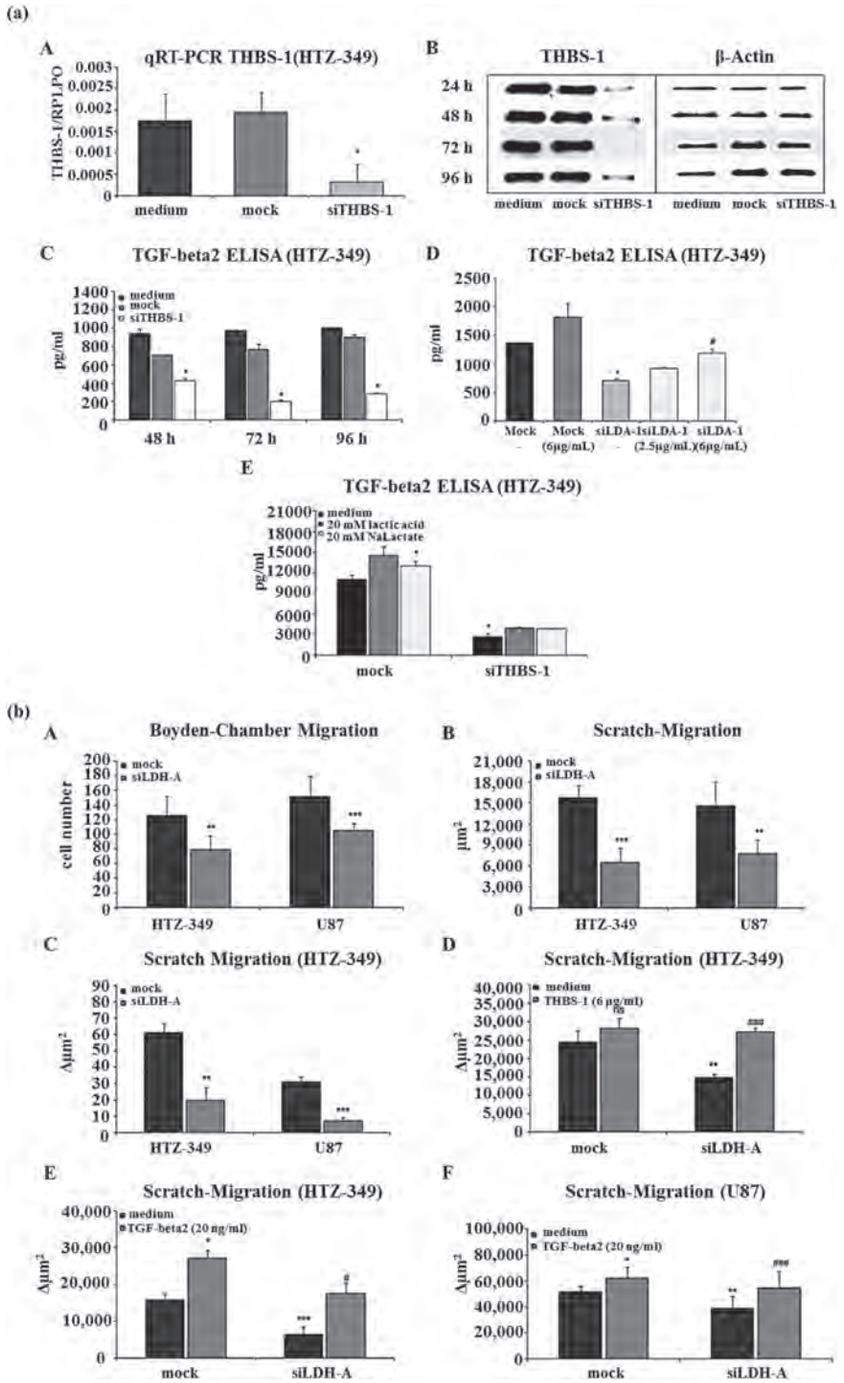

Figure 3. **Knockdown of THBS-1 down-regulates TGF-β2 at the protein level (a). siTHBS-1 significantly inhibit THBS-1 at the mRNA level (A, $p < 0.05$*). Western Blot analysis showed markedly reduced THBS-1 protein levels (B). In TGF-β2 ELISAs siTHBS-1 causes significant down-regulation of TGF-β2 protein in HTZ-349 glioma cells (C, $p < 0.05$*). Decreased levels of TGF-β2 protein after LDH-A knockdown (D, mock/siLDH-A $p < 0.05$*) can be rescued by addition of increasing doses of synthetic THBS-1 protein. Lactic acid and sodium lactate fail to significantly induce TGF-β2 expression after transfection with siTHBS-1 (E, $p < 0.05$*). Glioma cell migration is mediated by THBS-1 and TGF-β2 (b). Boyden Chamber assays of HTZ-349 and U87 glioma cells 24 hours after treatment with 0.1 μM siLDH-A show a significant inhibition of migration (A, U87 $p < 0.001$***; HTZ-349 $p < 0.01$**). Scratch Migration assays verified these results (B–F). Inhibition of LDH-A by siRNA yields similar results (B) as in the Boyden chamber assay. THBS-1 knockdown also diminishes HTZ-349 and U87 migration (C, HTZ-349 $p < 0.01$**; U87 $p < 0.001$***). Addition of 6 μg/ml recombinant THBS-1 (D) and 20 ng/ml TGF-β2 (E, F) can fully rescue impaired migration after LDH-A knockdown. Reprinted with permission from [141], C. Seliger, et al., Lactate-modulated induction of THBS-1 activates transforming growth factor (TGF)-beta 2 and migration of glioma cells *in vitro*. *PloS One* 8, 70 (2013). © 2013, Public Library of Science.**

for the exploitation of potential therapeutic strategies for glioblastoma therapy.

RNAi Toward Immune-Related Cells

The central nervous system (CNS) has traditionally been believed as immunologically privileged, which derived from

(i) a paucity of native antigen presenting cells (APCs) in the CNS;

(ii) limitations of leukocyte entry imposed by the blood-brain barrier (BBB);

(iii) absence of native T cells in the CNS and

(iv) the observation that tissues engrafted into the CNS are rejected more slowly than those grafted to other sites, implying that the immune system is inactive in the CNS and fails to interact effectively with the systemic immune system.[143–145]

Now, much more research confirms that the CNS is immunocompetent than immune-privileged and interacts dynamically with the systemic immune system.[146, 147] Researches focus on immune related-cells to modulate the immune microenvironment of glioma have widely reported.

Myeloid Cells

The high infiltration rate of tumor-associated-myeloid cells (TAMs) including microglial cells and tumor-associated macrophages that accumulate in the tumor mass is considered as a striking feature of glioblastoma.[148–150] Of note, it is now identified that the glioblastoma tumors are notoriously immunosuppressive and can manipulate myeloid cells to support tumor progression. To be specifically, those pro-tumorigenic effects of tumor-associated myeloid cells promote angiogenesis as well as tumor cell invasion, proliferation as well as survival.[151] It is critical to destroy tumor cells by presenting tumor-associated antigens (TAA) to immune system thereby generate tumor-specific immunity.[152–155] Among this process, APCs play a key role in harnessing the immune system to fight glioblastoma. As reported, microglia (MG),[156–159] macrophages (MP)[160–163] and dendritic cells[164–166] acted as powerful immune cells in the CNS.

Microglia (MG) is the resident immune cells of the CNS that responses to neuroinflammation by secreting proinflammatory cytokines as well as producing phagocytosing cell debris and pathogens.[167–169] The accumulation of MG and MP, which have been demonstrated in a number of CNS diseases, processes infection, trauma and neoplasia.[170] Kaminska et al. had shown that using plasmid-transcribed small hairpin RNAs (shRNAs) could reduce the level of TGF-β type II receptor (TβIIR) mRNA by 30%$\sim$67% in cells expressing specific shRNA. And siRNA-mediated knockdown of TβRII expression abrogated glioma growth in nude mice (Fig. 4). All those results indicated a critical role played by microglia-derived TGF-β in regulating tumor-host interactions.[171]

Recently, it had been widely shown that the activated microglia exhibited an immunosuppressive M2 phenotypes, which could promote the growth of gliomas. Eyupoglu and co-workers demonstrated that the inhibition of MIF (migration inhibitory factor, which results in a M2 shift of microglial cells) signaling through siRNA-mediated knockdown, could efficiently abrogate the tumor escape mechanism and foster antitumor activity (Fig. 5).[172] Those studies indicates that it is a viable approach to modulate the TME of glioma through shifting microglial cells from M2 toward M1 phenotypes thereby augment the treatment effect (Fig. 6).[172]

The antigen presentation cells, such as dendritic cells (DC), have also been confirmed among immune cell infiltrates in CNS tumors, and can modulate the immunity of host body.[173–175] To achieve proper immune responses by host for tumor treatments, antigen presentation by dendritic cells (DCs) is essential.[176–182] DCs can recognize and capture antigen in their immature state and then migrate to lymphoid organs and present processed peptides.[183–186] Kim and co-workers reported that down-regulation of PTEN (phosphatase and tensin homologue, a central negative regulator of the PI3K/AKT signal transduction cascade) in DCs resulted in an increase of *in vitro* T cell activation activity and *in vivo* migration to a draining lymph node.[187] Yu et al. found that transfection of DC with IL-10-specific double strands of small interference RNA (siRNA) could significantly enhance allogeneic T cell proliferation and promoted Th1 responses by increasing IFN-γ and decreasing IL-4 production. These findings collectively indicates the potential of using IL-10 siRNA transfected antigen-presenting cells for a novel immunotherapeutic strategy to elicit Th1 response.[188] In 2015, Kim and co-workers reported that the siRNA cocktail-mediated strategy by co-targeting immunosuppressive molecule, interleukin 10 receptor (siIL-10RA) and transforming growth factor-β receptor (siTGF-βR) on dendritic cells, could generate strong tumor antigen-specific $CD8^+$ T cell immunity (Fig. 7).[189]

Astrocytes

As previous reported, astrocytes form gap junctions with glioma cells.[190–194] The major connexin 43 (Cx43), which is expressed in both astrocytes and glioma cells, is specifically upregulated in the reactive astrocytes surrounding glioma, indicating that the interaction between glioma cells and surrounding astrocytes at the tumor margins are closely involved in glioma invasion (Fig. 8).[195–198] By manipulating gap junctions with a gap junction inhibitor, siRNAs, and a dominant negative connexin mutant, we showed that functional glioma-glioma gap junctions suppress glioma invasion while glioma-astrocyte and astrocyte-astrocyte gap junctions promote it in an *in vitro* transwell invasion assay.[191]

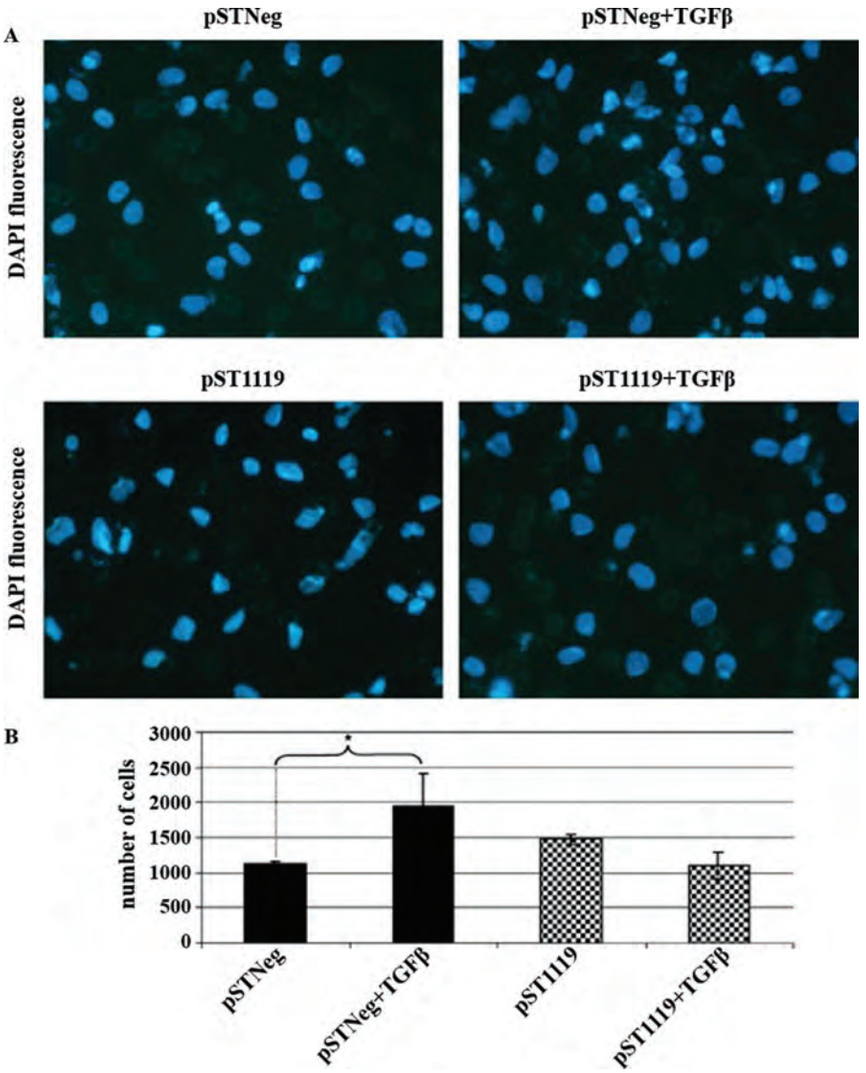

Figure 4. **TGF-β-induced invasiveness of T98G glioblastoma cells is reduced by shRNA against TβRII. (a) T98G cells transfected with pST1119 or pSTNeg plasmids were detached by trypsinization 24 h after transfection, collected by centrifugation and counted. Equal number of cells was seeded in 24-well transwell coated with the Growth Factor Reduced Matrigel Matrix and cultured in DMEM/2% FBS in the presence or absence of 5 ng/ml TGF-β1 for 24 h. The cells invading through Matrigel Matrix were fixed in ethanol, stained with DAPI and counted using a fluorescent microscope. Fluorescent images of stained cell nuclei from a representative experiment are shown. (b) Bar graphs show the number of invading cells among pST1119 or pSTNeg-transfected cells in the absence or presence of TGF-β1 (5 ng/ml). A statistically significant increase of Matrigel invading cells was observed after TGF-β treatment in pSTNeg-transfected cells (*$P < 0.05$ when compared to untreated cells). Data are presented as mean counts of cells in the entire microscopic field from duplicate wells in the representative experiment. Similar results were observed in three independent experiments. DAPI, 4′,6-diamidino-2-phenylindole, dihydrochloride; DMEM, Dulbecco's modified essential medium; FBS, fetal bovine serum; TGF-β, transforming growth factor-β; TβaRII, TGF-β type II receptor; shRNA, small hairpin RNA. Reprinted with permission from [171], A. Wesolowska, et al., Microglia-derived TGF-beta as an important regulator of glioblastoma invasion—An inhibition of TGF-beta-dependent effects by shRNA against human TGF-beta type II receptor. *Oncogene* 27, 918 (2008). © 2008, Nature Publishing Group.**

T Lymphocytes

T lymphocytes ($CD3^+$) fall into two major categories: $CD4^+$ T helper (Th) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) in addition to Tregs.[199–202] It is generally believed that high levels of $CD8^+$ CTLs are associated with a greater anti-tumor activity, while high levels of Tregs cells are regarded as being related to promote tumor progression.[203, 204] The induction and/or maintenance of immunosuppression in glioblastoma is partly due to the infiltration and accumulation of the highly immunosuppressive regulatory T cells (Treg).[52, 63, 205–207]

Glioma progression depends on the rapid proliferation of tumor cells accompanied by an acute immunosuppressive environment. Lesniak et al. reported that by

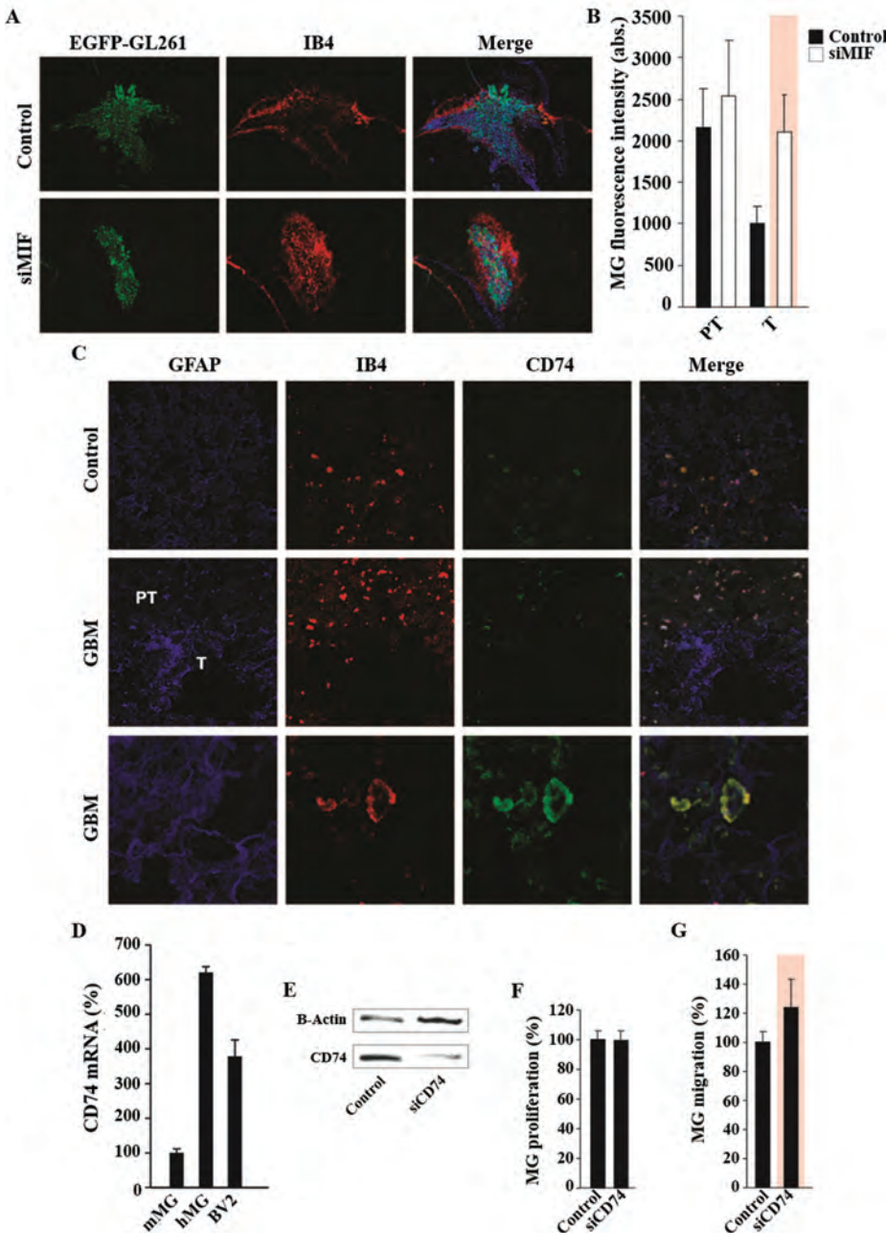

Figure 5. **Glioma derived MIF activates microglial CD74. (a) Brains of wild-type glioma implanted (control) and MIF knockdown implanted gliomas (siMIF) were cryo-sectioned and analyzed for glioma growth and microglia infiltration. Upper row shows wild-type glioma expansion (green), microglial distribution (red) and nuclei staining (blue). Lower panel shows microglial distribution in gliomas with siRNA-silenced MIF. Scale bar, 3 mm. (b) Quantification of microglial cells in peritumoral region (PT), and tumor bulk (T) in wild-type gliomas (black columns) and MIF knocked down gliomas (white columns) with $n = 4$. (c) Human brain tissues were cryo-sectioned and analyzed for microglial distribution. Human microglial cells are accumulated in peritumoral regions and CD74$^+$in glioblastoma affected brain tissue. Upper panel shows representative image of astroglial distribution (blue, GFAP), microglial cells (red, IB4) and CD74 expression (green) in human brain tissue (nonmalignant brain tissue, control). Right panel gives merged images of each row. Microglial cells (red, IB4$^+$) are accumulated in peritumoral regions and co-localized with CD74 (green) in human brains diagnosed for glioblastoma (GBM, revealed by GFAP immunostaining, blue). Lower panel, higher magnification of CD74 (green) positive microglial cells (IB4, red). Scale bar in middle column represents 70 μm and in lower column 5 μm. (d) Expression of CD74 in primary murine, human and BV2 microglia was analyzed by quantitative RT–PCR. (e) Expression of CD74 in BV2 microglial cells (control) and BV2 microglial cells with siRNA-mediated CD74 knockdown (siCD74) revealed by representative immunoblotting. β-Actin serves as a loading control. (f) Microglial proliferation is not affected, (g) whereas siRNA-mediated CD74 silencing in microglial cells alleviates migration. The experiments were repeated three times with $n = 12$. Reprinted with permission from [172], A. Ghoochani, et al., MIF-CD74 signaling impedes microglial M1 polarization and facilitates brain tumorigenesis. *Oncogene 35*, 1 (2016). © 2016, Nature Publishing Group.**

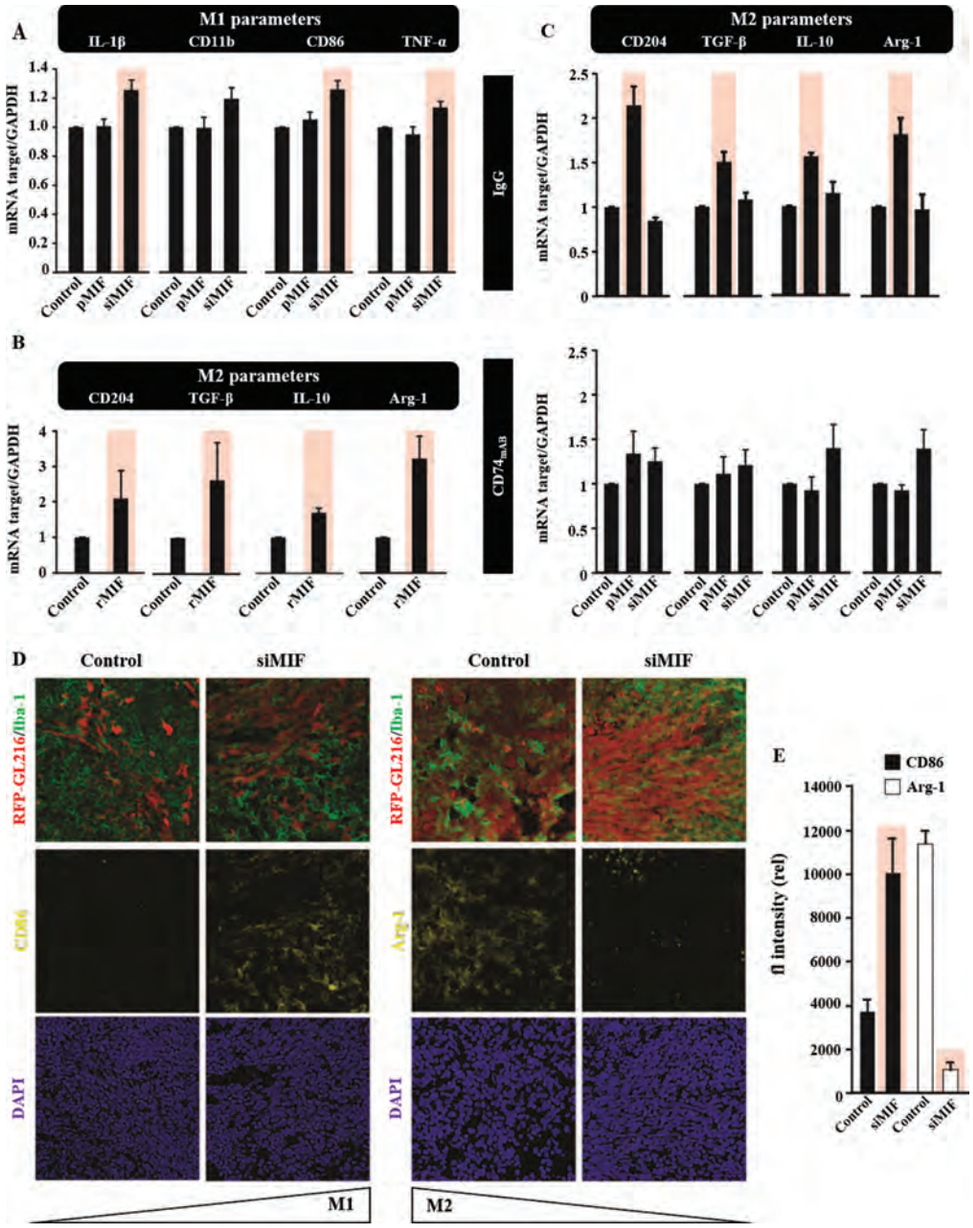

Figure 6. **MIF shifts microglia into a M2 phenotype. (a) Analysis of M1 parameters in co-cultivated BV2 microglia cells with genetically modified GL261 glioma cells ($n = 3$). Co-cultures contain a semi-permeable membrane, which separates both cell entities. After 20 h co-culture BV2 microglia were analyzed for expression of M1 markers by quantitative RT-PCR. Almost all M1 parameters significantly increase in their expression in BV2 microglia after co-culture with MIF over-secreting GL261 glioma cells, whereas siRNA-silenced GL261 glioma cells do not. (b) M2 markers in primary murine microglia were analyzed by quantitative RT–PCR after rMIF treatment ($n = 3$). 20 h of rMIF treatment significantly increases expression of M2 parameters (that is, CD204, TGF-β, IL-10 and Arg-1). (c) Analysis of M2 parameters in co-cultivated BV2 microglia cells with genetically modified GL261 glioma cells ($n = 3$). Co-cultures contain a semi-permeable membrane, which separates both cell entities. After 20 h co-culture BV2 microglia were analyzed for expression of M2 markers by quantitative RT–PCR. M2 parameters significantly increase in their expression in BV2 microglia after co-culture with MIF over-secreting GL261 glioma cells, whereas siRNA-silenced GL261 glioma cells do not. (d) Analysis of microglial shift *in vivo*. Brain sections of GL261 (red) implanted mice were analyzed for expression of the M1 and M2 markers (yellow) CD86 and Arg-1. Nuclei were stained by DAPI (blue). Total microglia staining was performed by Iba-1 staining (green). Left column, CD86 as a representative marker for M1 polarization was dramatically increased in MIF-silenced gliomas compared with wild-type MIF-expressing glioma cells. Right column, Arg-1 as a representative marker for M2 polarization was conversely decreased in MIF silenced gliomas compared with wild-type MIF-expressing gliomas. Scale bar, 20 μm. (e) Quantitative analysis of CD86 (black) and Arg-1 (white) staining through measurement of fluorescence intensities. Reprinted with permission from [172], A. Ghoochani, et al., MIF-CD74 signaling impedes microglial M1 polarization and facilitates brain tumorigenesis. *Oncogene 35*, 1 (2016). © 2016, Nature Publishing Group.**

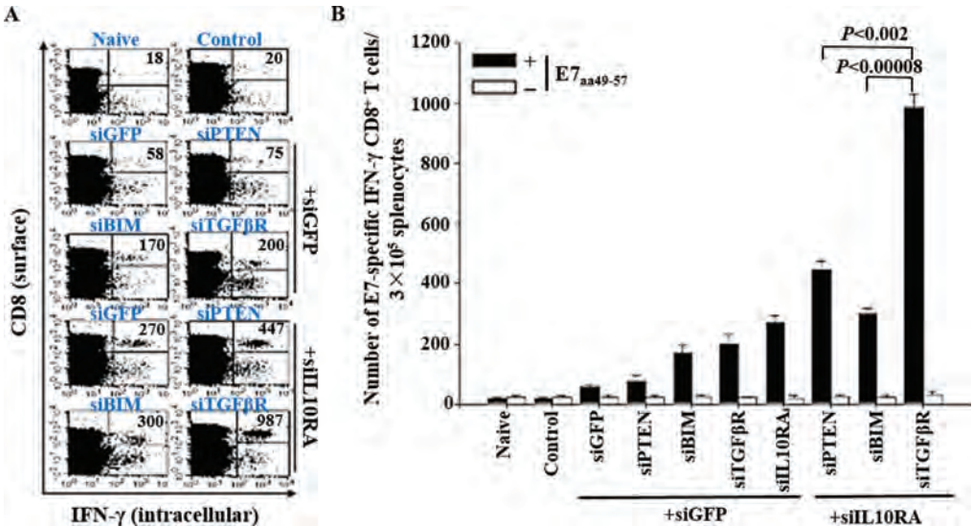

Figure 7. **Vaccination with dendritic cells (DCs) transfected with various small interfering RNA (siRNA) increases the number of E7-specific CD8$^+$ T cells. (a) Intracellular cytokine staining and flow cytometry analysis to determine the number of interferon (IFN)-γ-producing E7-specific CD8$^+$ T cells in mice after immunization with E7 peptide-pulsed DCs transfected with various siRNA constructs. Mice (five per group) were vaccinated twice with E7 peptide-pulsed DCs transfected with siRNA targeting green fluorescent protein (GFP), phosphatase and tensin homologue deleted on chromosome 10 (PTEN), Bcl-2-like protein 11 (BIM) interleukin (IL)-10RA, transforming growth factor (TGF)-βR, IL-10RA1PTEN, IL-10RA1BIM or IL-10RA1TGF-βR. There was a 1-week interval between injections of the transfected DCs for the purpose of vaccination. Splenocytes were harvested 1 week after the last vaccination, stained for CD8$^+$ and IFN-γ, and analysed by flow cytometry to detect activated E7-specific CD8$^+$ T cells. Representative flow cytometry data for splenocytes harvested from the vaccinated mice and stimulated with E7 aa49-57 peptide or without peptide stimulation. The naive group has non-transfected DCs without E7 peptide pulsing, while the control group has siGFP transfected DC without E7 peptide pulsing. (b) The bar graph indicates the number of IFN-γ-expressing E7-specific CD8$^+$ T cells per 3 x 10^5 splenocytes from vaccinated mice (mean 6 standard deviation). The data presented in this figure are representative of two independent experiments. Reprinted with permission from [189], Y. H. Ahn, et al., The siRNA cocktail targeting interleukin 10 receptor and transforming growth factor-receptor on dendritic cells potentiates tumour antigen-specific CD8$^+$ T cell immunity. *Clin. Exp. Immunol.* 181, 164 (2015). © 2015, Wiley-Blackwell.**

using short hairpin RNA to silencing of gene expression, the expression of fibronectin in glioma cells could be inhibited, and cell proliferation delayed *in vitro*.[208] In animal model, the level of FoxP3 expression by CD4$^+$ T cells in GL261-FnKD-implanted brains at 14th day was almost 2.2-fold lower than that in GL261-VC tumors. After 21 days, the difference was increased to almost 2.6-fold with 63.4% of CD4$^+$ T cells expressing FoxP3 in GL261-VC tumors, whereas 24.8% CD4$^+$ T cells expressed FoxP3 in GL261-FnKD tumors. Besides, brain samples of animals bearing fibronectin-knockdown tumors showed delayed Treg recruitment. All those data suggest that the knockdown of fibronectin expression can prepare a reduced immunosuppressive environment.

The lactose binding lectin Galectin-1 (Gal-1), which is overexpressed in GBM, have an immunosuppressive and chemo- and immunotherapy resistance properties in tumor.[209] Rubinstein et al. found that inhibition of Gal-1 induced a generation of the tumor-specific Th1-type immune response *in vivo*.[210] In 2017, an investigation of the consequences of reducing Gal-1 in the TME during the GBM progression on both myeloid and lymphoid compartments of the immune system was conducted by Matthias Van Woensel and co-workers. The results shown that the reduction of Gal-1 could induce an alleviation from the immune suppression, while increasing the immune activation (Fig. 9).[211]

Natural Killer (NK) Cells

Natural killer (NK) cells were originally defined as effector lymphocytes of innate immunity endowed with constitutive cytolytic functions.[212–214] Natural killer cells are part of the innate immune defense system which serve as a very important role in host defense through apoptotic killing of tumor cells.[215–217] Due to the lack of antigen-specific cell surface receptors, they are generally considered as components of innate immune defense. Recent reports have clarified that NK cells presented within humans and mice participated in the early control against virus infection, especially in tumor immune-surveillance.[218] It is generally considered that NKG2D operates as an activating receptor on natural killer (NK) cells and mediates tumor immune surveillance. The induction of stress-inducible ligands for the activatory NK cell receptor NKG2D may prompt early recognition of transformed cells. Using siRNA-mediated silencing of HLA-E, which had an immune-inhibitory effect on tumor-specific CTL, enabled NKG2D-mediated lysis of Ref. [51] Cr-labeled tumor cells by NK cells.

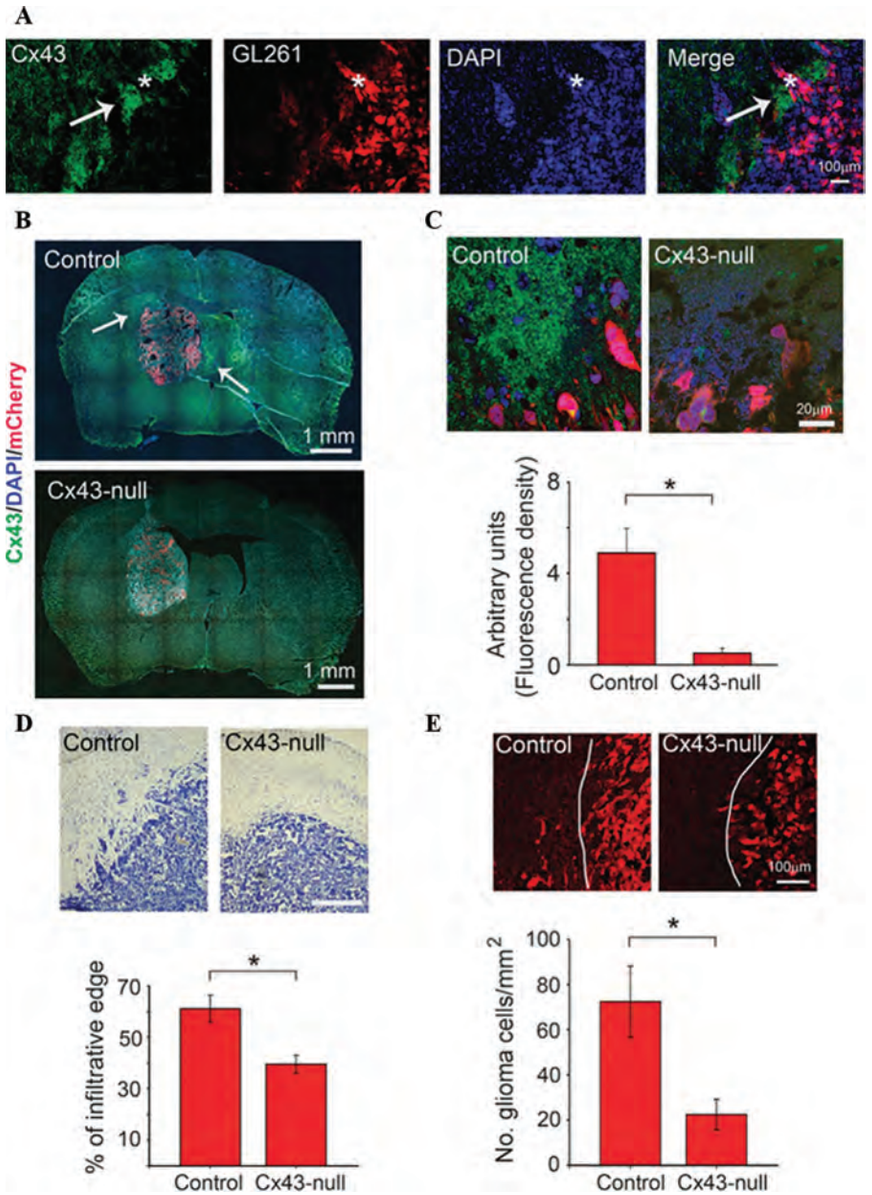

Figure 8. **Astrocytic Cx43 promotes dissemination of glioma cells. (A) Increased Cx43 staining (white arrow) adjacent to GL261 cells (white asterisk) that were protruding into the brain parenchyma. Nuclei were stained blue with 4′-6-diamidino-2-phenylindole (DAPI). (B) Low magnification overview of control ($Cx43^{fl/fl}$) and Cx43-null (Nestin-Cre:$Cx43^{fl/fl}$) brains implanted with GL261 glioma cells (red) showing Cx43 (green) expression in control brains. Nuclei were stained blue with DAPI. White arrows indicate region with increased Cx43 expression. (C, Upper) Magnified image showing expression of Cx43 (green) adjacent to GL261 glioma cells (red) in control ($Cx43^{fl/fl}$) but not in Cx43-null (Nestin-Cre:$Cx43^{fl/fl}$) brains. Nuclei were stained blue with DAPI. (Lower) Semi-quantitative analysis of Cx43 immunoreactivity at the peri-tumor by Image J. *$P < 0.001$ (Mann-Whitney rank-sum test). (D) Cresyl violet-stained glioma sections showing circumscribed border in the Cx43-null brain compared with the control. Scale bar = 200 μm. Increased percentage of tumor border with infiltrative cells in control ($n = 6$) compared with Cx43-null brains ($n = 5$). *Po0.05 (Student's *t*-test). (e) Increased number of mCherry-positive GL261 glioma cells in the brain parenchyma adjacent to the glioma border (white line) in control wild-type ($n = 5$) compared with Cx43-null brains ($n = 5$). *$P < 0.05$. Data are shown as mean ± s.e.m. Reprinted with permission from [197], W. C. Sin, et al., Astrocytes promote glioma invasion via the gap junction protein connexin43. *Oncogene* 35, 1504 (2016). © 2016, Nature Publishing Group.**

Lectin-like transcript-1 (LLT1), which is expressed on cells of lymphocytic origin, is a newly identified ligand for the inhibitory natural killer (NK) cell receptor CD161. LLT1 contributes to tumor-associated immunosuppression by affecting the lytic activity of NK cells. Weller et al. reported that small interfering RNA (siRNA)-mediated down-regulation of LLT1 in LNT-229 and LN-428 cells could promote their lysis by NK cells.[219]

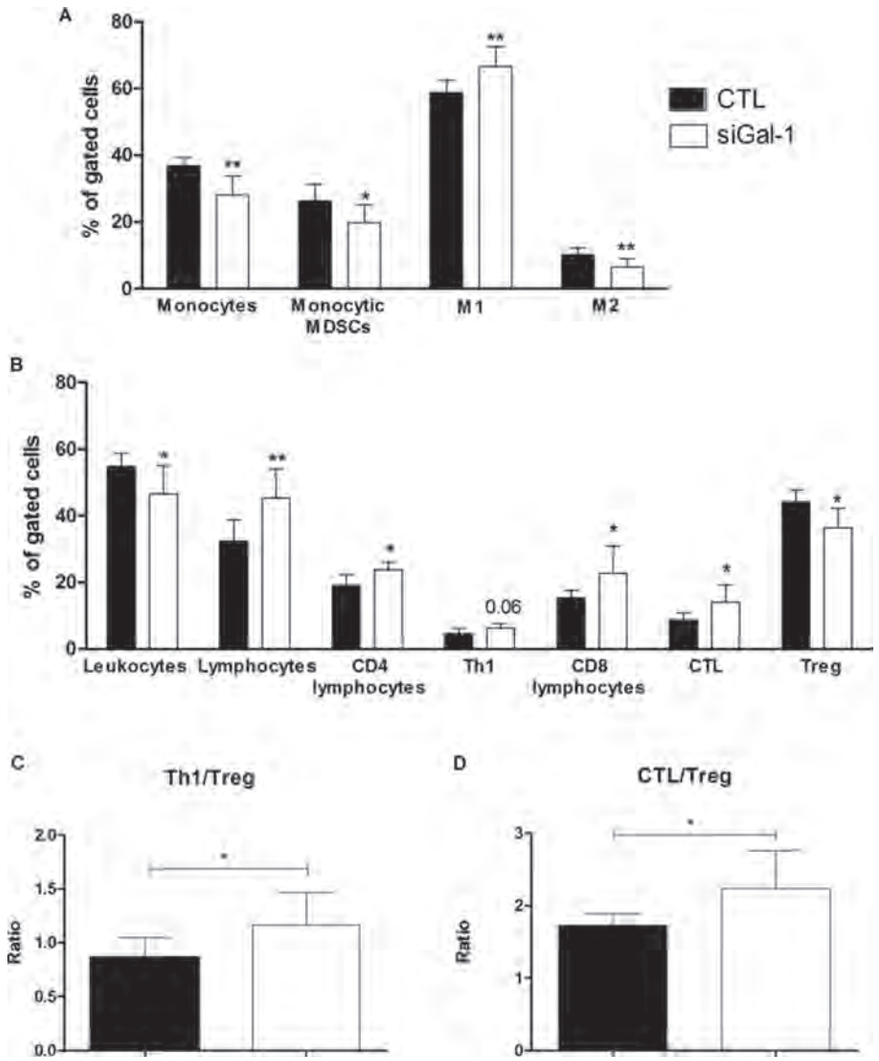

Figure 9. **siGal-1 alleviates immune suppression and induces immune activation during GBM progression. Flow cytometry was performed on isolated mononuclear brain infiltrating cells of mice that were left untreated, or treated with siGal-1 on day 4, 8, 12 and 15 after tumor inoculation, and brains were isolated at day 20. Different stainings assess several cell populations with (A) the myeloid cells, with monocytes as gated by ZY^-, $CD45^+$, $CD11b^+$; monocytic MDSCs as ZY^-, $CD45^+$, $CD11b^+$, $Ly6C^+$; M1 macrophage phenotype as $CD45^+$, $CD11b^+$, ZY^-, MRC^-, $MHCII^{high}$; M2 macrophage phenotype as $CD45^+$, $CD11b^+$, ZY^-, MRC^+, $MHCII^{low}$. (B) The lymphoid cells with leukocytes, as single cells, ZY^-, $CD45^+$; lymphocytes as single cells, ZY^-, $CD45^+$, $CD3^+$, CD4 lymphoctyes as single cells, ZY^-, $CD45^+$, $CD3^+$, $CD4^+$; T1 as single cells, ZY^-, $CD45^+$, $CD3^+$, $CD4^+$, IFNγ^+ gated to $CD45^+$; CD8 lymphocytes as single cells, ZY^-, $CD45^+$, $CD3^+$, $CD8^+$; CTL as single cells, ZY^-, $CD45^+$, $CD3^+$, $CD8^+$, IFNγ^+ gated to CD45; Tregs, as gated by single cells, ZY^-, $CD45^+$, $CD3^+$, $CD4^+$, $FoxP3^+$; (C) the ratio immune activation to immune suppression was calculated for T1 (IFNγ^+ $CD4^+$ $CD3^+$ $CD45^+$ ZY^-) and (D) CTL (IFNγ^+ $CD8116^+$ $CD3^+$ $CD45^+$ ZY^-), as compared to Treg ($FoxP3^+$ $CD4^+$ $CD3^+$ $CD45^+$). White bars represent the siGal-1 treated mice, black bars are control tumor bearing mice and groups were compared with unpaired *t*-test ($n = 5/10$ per group, $^*p < 0.05$, $^{**}p < 0.01$). Reprinted with permission from [211], M. Van Woensel, et al., Sensitization of glioblastoma tumor micro-environment to chemo- and immunotherapy by Galectin-1 intranasal knock-down strategy. *Sci. Rep.* 7, 1217 (2017). © 2017, Scientific Reports.**

Therapeutic approaches directly focus on immune cells exhibit better behavior against glioblastoma. Through modulating the performance of the immune cells, the immune microenvironment can be regulated and the anti-tumor efficiency can be enhanced. However, the therapeutic effect is insufficient by single factor immune modulation due to the complex immune environment of glioblastoma. As previously reported, a variety of target genes associated with suppressive tumor microenvironment played important roles in the immune regulation of the tumor microenvironment (Table I).[220] Collectively, combination of RNAi-based immunomodulation with other therapeutics need to explore for efficient treatment of glioblastoma.

Table I. Target genes in the suppressive tumor microenvironment.

Gene/protein	Target cell(s)	Strategy
CCL2	TAMs	siRNA to ↓ expression
IL-6	TAMs	siRNA to ↓ expression
Dominant negative CCL2 mutant gene (7ND)	TAMs	Gene transfer to ↑ expression
CCL16	TAMs	Gene transfer to ↑ expression
IL-10	TAMs, Tregs	siRNA to ↓ expression
Heme oxygenase-1 (HO-1)	Tregs	siRNA to ↓ expression
Transforming growth factor-β (TGF-β)	TAMs, MDSC, Tregs	siRNA to ↓ expression
STAT1	MDSC	siRNA to ↓ expression
Indoleamine 2,3-dioxygenase (IDO)	APCs	siRNA to ↓ expression
IL-6, IL-8, CCL22	Tumor (with goal to prevent TAM recruitment)	siRNA to ↓ expression
CXCL12, SDF-la, CCL2, CXCL5, KIT ligand, IL-1β GM-CSF, VEGF	Tumor (with goal to prevent MDSC recruitment)	siRNA to ↓ expression
CCL5	Tumor (with goal to prevent Treg recruitment)	siRNA to ↓ expression

Source: Reprinted with permission from [220]. E. A. Vasievich and L. Huang, The suppressive tumor microenvironment: A challenge in cancer immunotherapy. *Mol. Pharmaceut.* 8, 635 **(2011)**. © 2011, American Chemical Society.

STRATEGIES COMBING WITH IMMUE MICROENVIRONMENT MODULATION

To achieve optimal treatment efficiency, different therapy strategies coupled with immunotherapy, especially immunomodulation, are now be used. For example, the main determinant of the resistance to the alkylating agent temozolomide (TMZ) is O^6-methylguanine-DNA methyltransferase (MGMT). The downregulation of MGMT seems improve the sensitivity of glioma to TMZ.[221] In 2010, Kato et al. used a novel liposome (LipoTrust EX Oligo) to delivery small-interfering RNA (siRNA for MGMT) into GBM-initiating cells (GICs). The knockdown of MGMT expression was investigated by using an MGMT-siRNA/LipoTrust complex. MGMT was expressed in 99% of the tumor cells administered with control siRNA, compared with only 7% of cells in MGMT-siRNA-administered tumors. More importantly, treatment of subcutaneous tumor models with a combination of MGMT-siRNA and TMZ showed a significant decrease in tumor growth (<2500 mm^3), while mice that were administered TMZ or siRNA alone showed slight tumor reduction ($>5000/10000$ mm^3, respectively).[222]

Similarly, strategy to improve the radio-sensitivity of glioma cells is a feasible solution to augment the efficacy of radiotherapy. It is reported that signal transducer and activator of transcription 3 (STAT3) inhibition could enhance the sensitivity of radiotherapy in glioma.[223] In 2015, Liu and colleagues found that inhibition of STAT3 signaling as well as autophagy could further increase the radio-sensitivity of glioma cells.[224]

Although current immunomodulatory drugs and therapies for treating brain tumors have been discussed, new and potentially groundbreaking strategies are on the horizon. In addition, in order to maximize therapeutic benefit, systematic investigation of potential variables that may impact the optimal activity of immunotherapies and the evaluation of potentially informative biomarkers will be required.

CONCLUSIONS AND PERSPECTIVES

In this review, we summary the characters of glioma related microenvironment and the current therapeutics by using RNAi technology. Two components are major "culprits" which promote the immunosuppression microenvironment. One is the feather of tumor cell, including secreted cytokines and surface molecules; another issue is the involved surrounding cells, such as APC, DC, T cells and so on, as well as signaling pathway. Based on those theoretical foundations and experimental results, it is a great promising strategy to modulate the immune microenvironment and enhance the efficiency of anti-glioblastoma therapy by utilizing RNAi technology.

Considering the complex brain tumor microenvironment, optimized combination strategies are urgently needed to obtain great effective therapy, including cytotoxic (chemotherapy, radiotherapy) and immune-stimulatory approaches (immunomodulation). For example, different siRNA with multiple targets can be utilized into one treatment system. Those synergetic strategies collectively enhance the efficacy of glioblastoma treatment via accurately silencing related protein expression.

Nevertheless, using RNAi to improve the immune microenvironment remains several challenges. Firstly, highly specific targeting should be exactly executed *in vivo*, thus avoiding off-target effects. Secondly, more stable and potent RNAi systems play an important role in fulfilling higher gene suppression following by a success tumor immunomodulation. With the deeper understanding of immune mechanisms involving the CNS/glioma immunobiology, more immunological targets are identified, and further relationships between glioblastoma and immune microenvironment are explored. All of these will lead us the way for the development of powerful combination therapies against glioblastoma and other brain tumors.

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REFERENCES

1. T. A. Dolecek, J. M. Propp, N. E. Stroup, and C. Kruchko, CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2005–2009. *Neuro. Oncol.* 14, v1 **(2012)**.
2. S. Ilkanizadeh, J. Lau, M. Huang, D. J. Foster, R. Wong, A. Frantz, S. Wang, W. A. Weiss, and A. I. Persson, Glial progenitors as targets for transformation in glioma. *Adv. Cancer Res.* 121, 1 **(2014)**.
3. M. Preusser, S. de Ribaupierre, A. Wohrer, S. C. Erridge, M. Hegi, M. Weller, and R. Stupp, current concepts and management of glioblastoma. *Ann. Neurol.* 70, 9 **(2011)**.
4. J. Aparicio-Blanco and A. I. Torres-Suarez, Glioblastoma multiforme and lipid nanocapsules: A review. *J. Biomed. Nanotechnol.* 11, 1283 **(2015)**.
5. A. Bronisz, Y. Wang, M. O. Nowicki, P. Peruzzi, K. I. Ansari, D. Ogawa, L. Balaj, G. De Rienzo, M. Mineo, I. Nakano, M. C. Ostrowski, F. Hochberg, R. Weissleder, S. E. Lawler, E. A. Chiocca, and J. Godlewski, Extracellular vesicles modulate the glioblastoma microenvironment via a tumor suppression signaling network directed by miR-1. *Cancer Res.* 74, 738 **(2014)**.
6. H. B. Wang, Z. Hua, D. Z. Ma, and L. Tao, MiR-217 promoted the proliferation and invasion of glioblastoma by repressing YWHAG. *Cytokine* 92, 93 **(2017)**.
7. P. O. Zinn, S. K. Singh, and R. R. Colen, Shedding light on glioblastoma cellular heterogeneity. *Neuro-Oncol.* 17, 327 **(2015)**.
8. D. K. Zhang, F. Wang, Y. Pang, E. H. Zhao, S. Q. Zhu, F. Chen, and H. J. Cui, ALG2 regulates glioblastoma cell proliferation, migration and tumorigenicity. *Biochem. Bioph. Res. Co.* 486, 300 **(2017)**.
9. Y. Yin, K. J. Ornell, A. Beliveau, and A. Jain, Modulation of MicroRNAs 34a and 21 affects viability, senescence, and invasion in glioblastoma multiforme. *J. Biomed. Nanotechnol.* 12, 1782 **(2016)**.
10. S. A. Grossman, X. B. Ye, S. Piantadosi, S. Desideri, L. B. Nabors, M. Rosenfeld, J. Fisher, and N. C. Consortium, Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. *Clin. Cancer Res.* 16, 2443 **(2010)**.
11. M. Candolfi, K. M. Kroeger, A. K. M. G. Muhammad, K. Yagiz, C. Farrokhi, R. N. Pechnick, P. R. Lowenstein, and M. G. Castro, Gene therapy for brain cancer: Combination therapies provide enhanced efficacy and safety. *Curr. Gene Ther.* 9, 409 **(2009)**.
12. J. F. Mineo, A. Bordron, M. Baroncini, C. Ramirez, C. A. Maurage, S. Blond, and P. Dam-Hieu, Prognosis factors of survival time in patients with glioblastoma multiforme: A multivariate analysis of 340 patients. *Acta Neurochir.* 149, 245 **(2007)**.
13. M. J. B. Taphoorn, R. Stupp, C. Coens, D. Osoba, R. Kortmann, M. J. van den Bent, W. Mason, R. O. Mirimanoff, B. G. Baumert, E. Eisenhauer, P. Forsyth, A. Bottomley, and N. Ctg, Health-related quality of life in patients with glioblastoma: A randomised controlled trial. *Lancet Oncol.* 6, 937 **(2005)**.
14. P. Y. Wen, and S. Kesari, Malignant gliomas in adults. *New. Engl. J. Med.* 359, 492 **(2008)**.
15. S. E. L. Craig, J. Wright, A. E. Sloan, and S. M. Brady-Kalnay, Fluorescent-guided surgical resection of glioma with targeted molecular imaging agents: A literature review. *World Neurosurg.* 90, 154 **(2016)**.
16. G. D'Andrea, P. Familiari, A. Di Lauro, A. Angelini, and G. Sessa, Safe resection of gliomas of the dominant angular gyrus availing of preoperative FMRI and intraoperative DTI: Preliminary series and surgical technique. *World Neurosurg.* 87, 627 **(2016)**.
17. K. Messaoudi, A. Clavreul, and F. Lagarce, Toward an effective strategy in glioblastoma treatment. Part II: RNA interference as a promising way to sensitize glioblastomas to temozolomide. *Drug Discov. Today* 20, 772 **(2015)**.
18. Y. I. Jeong, Y. W. Kim, S. Jung, J. Pei, M. Wen, S. Y. Li, H. H. Ryu, J. C. Lim, W. Y. Jang, I. Y. Kim, K. S. Moon, and T. Y. Jung, Delivery of transferrin-conjugated polysaccharide nanoparticles in 9L gliosacoma cells. *J. Nanosci. Nanotechnol.* 15, 125 **(2015)**.
19. Y. Y. Xu, M. Shen, Y. Sun, P. Gao, and Y. R. Duan, Polymer nanocomposites based thermo-sensitive gel for paclitaxel and temozolomide co-delivery to glioblastoma cells. *J. Nanosci. Nanotechnol.* 15, 9777 **(2015)**.
20. Y. C. Wang, R. C. Liang, and F. Fang, Applications of nanomaterials in radiotherapy for malignant tumors. *J. Nanosci. Nanotechnol.* 15, 5487 **(2015)**.
21. Y. Y. Xu, M. Shen, Y. Sun, P. Gao, and Y. R. Duan, Nano composite thermo-sensitive gel for paclitaxel and temozolomide co-delivery to glioblastoma cells. *J. Nanosci. Nanotechnol.* 16, 12288 **(2016)**.
22. D. H. Shin, S. J. Lee, J. S. Kim, J. H. Ryu, and J. S. Kim, Synergistic effect of immunoliposomal gemcitabine and bevacizumab in glioblastoma stem cell-targeted therapy. *J. Biomed. Nanotechnol.* 11, 1989 **(2015)**.
23. L. Sun, D. Y. Joh, A. Al-Zaki, M. Stangl, S. Murty, J. J. Davis, B. C. Baunnann, M. Alonso-Basanta, G. D. Kao, A. Tsourkas, and J. F. Dorsey, Theranostic application of mixed gold and superparamagnetic iron oxide nanoparticle micelles in glioblastoma multiforme. *J. Biomed. Nanotechnol.* 12, 347 **(2016)**.
24. A. Q. Sugihara, C. E. Rolle, and M. S. Lesniak, Regulatory T cells actively infiltrate metastatic brain tumors. *Int. J. Oncol.* 34, 1533 **(2009)**.
25. A. Ksendzovsky, D. Feinstein, R. Zengou, A. Sharp, P. Polak, T. Lichtor, and R. P. Glick, Investigation of immunosuppressive mechanisms in a mouse glioma model. *J. Neuro-Oncol.* 93, 107 **(2009)**.
26. A. B. Heimberger, M. Abou-Ghazal, C. Reina-Ortiz, D. S. Yang, W. Sun, W. Qiao, N. Hiraoka, and G. N. Fuller, Incidence and prognostic impact of FoxP3(+) regulatory T cells in human gliomas. *Clin. Cancer. Res.* 14, 5166 **(2008)**.
27. A. El Andaloussi, and M. S. Lesniak, An increase in CD4+CD25+FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. *Neuro-Oncol.* 8, 234 **(2006)**.
28. B. M. Kerklaan, O. van Tellingen, A. D. R. Huitema, J. H. Beijnen, W. Boogerd, J. H. M. Schellens, and D. Brandsma, Strategies to target drugs to gliomas and CNS metastases of solid tumors. *J. Neurol.* 263, 428 **(2016)**.
29. F. Zhang, C. L. Xu, and C. M. Liu, Drug delivery strategies to enhance the permeability of the blood-brain barrier for treatment of glioma. *Drug Des. Dev. Ther.* 9, 2089 **(2015)**.
30. C. Y. Zhan and W. Y. Lu, The Blood-brain/tumor barriers: Challenges and chances for malignant gliomas targeted drug delivery. *Curr. Pharm. Biotechnol.* 13, 2380 **(2012)**.
31. Z. F. Jiang, X. F. Wang, Y. S. Zhang, P. L. Zhao, Z. X. Luo, and J. Li, Effect of capsaicin-loading nanoparticles on gliomas. *J. Nanosci. Nanotechnol.* 15, 9834 **(2015)**.
32. G. Salzano, S. Zappavigna, A. Luce, N. D'Onofrio, M. L. Balestrieri, A. Grimaldi, S. Lusa, D. Ingrosso, S. Artuso, M. Porru, C. Leonetti, M. Caraglia, and G. De Rosa, Transferrin-targeted nanoparticles containing zoledronic acid as a potential tool to inhibit glioblastoma growth. *J. Biomed. Nanotechnol.* 12, 811 **(2016)**.
33. S. Ostrand-Rosenberg, Tolerance and immune suppression in the tumor microenvironment. *Cell Immunol.* 299, 23 **(2016)**.

34. J. C. Becker, M. H. Andersen, D. Schrama, and P. T. Straten, Immune-suppressive properties of the tumor microenvironment. *Cancer Immunol. Immun.* 62, 1137 **(2013)**.

35. A. A. Hurwitz and S. K. Watkins, Immune suppression in the tumor microenvironment: A role for dendritic cell-mediated tolerization of T cells. *Cancer Immunol. Immun.* 61, 289 **(2012)**.

36. N. J. Abbott, A. A. K. Patabendige, D. E. M. Dolman, S. R. Yusof, and D. J. Begley, Structure and function of the blood-brain barrier. *Neurobiol. Dis.* 37, 13 **(2010)**.

37. A. K. Reinhold and H. L. Rittner, Barrier function in the peripheral and central nervous system—A review. *Pflug. Arch. Eur. J. Phycol.* 469, 123 **(2017)**.

38. P. Sminia and B. A. Westerman, Blood-brain barrier crossing and breakthroughs in glioblastoma therapy. *Brit. J. Clin. Pharmaco.* 81, 1018 **(2016)**.

39. X. H. Mao, Z. Y. Ren, B. Huang, M. M. Pu, H. J. Li, Y. N. Ding, Q. F. Xu, Y. H. Zhang, C. L. Zhang, Z. Lu, Y. X. Chen, and Y. P. Zhao, Daunorubicin loaded Fe_3O_4 nanoparticles induce apoptosis of glioma cells and disrupt tight junction at blood-brain barrier. *J. Nanosci. Nanotechnol.* 16, 12356 **(2016)**.

40. M. M. Patel and B. M. Patel, Crossing the blood-brain barrier: Recent advances in drug delivery to the brain. *Cns. Drugs* 31, 109 **(2017)**.

41. C. F. Cho, J. M. Wolfe, C. M. Fadzen, D. Calligaris, K. Hornburg, E. A. Chiocca, N. Y. R. Agar, B. L. Pentelute, and S. E. Lawler, Blood-brain-barrier spheroids as an *in vitro* screening platform for brain-penetrating agents. *Nat. Commun.* 8 **(2017)**.

42. C. Stegmayr, U. Bandelow, D. Oliveira, P. Lohmann, A. Willuweit, C. Filss, N. Galldiks, J. H. R. Lubke, N. J. Shah, J. Ermert, and K. J. Langen, Influence of blood-brain barrier permeability on O-(2-F-18-fluoroethyl)-L-tyrosine uptake in rat gliomas. *Eur. J. Nucl. Med. Mol. I* 44, 408 **(2017)**.

43. H. Peluffo, U. Unzueta, M. L. Negro-Demontel, Z. K. Xu, E. Vaquez, N. Ferrer-Miralles, and A. Villaverde, BBB-targeting, protein-based nanomedicines for drug and nucleic acid delivery to the CNS. *Biotechnol. Adv.* 33, 277 **(2015)**.

44. Z. Z. Yang, W. Gao, Y. J. Liu, N. Pang, and X. R. Qi, Delivering siRNA and chemotherapeutic molecules across BBB and BTB for intracranial glioblastoma therapy. *Mol. Pharmaceut.* 14, 1012 **(2017)**.

45. K. Kingwell, Drug delivery new targets for drug delivery across the BBB. *Nat. Rev. Drug Discov.* 15, 84 **(2016)**.

46. Z. Y. Yin, T. T. Yu, and Y. Xu, Preparation of amyloid immunonanoparticles as potential MRI contrast agents for alzheimer's disease diagnosis. *J. Nanosci. Nanotechnol.* 15, 6429 **(2015)**.

47. J. H. Jeong, J. J. Kim, D. H. Bak, K. S. Yu, J. H. Lee, N. S. Lee, Y. G. Jeong, D. K. Kim, D. K. Kim, and S. Y. Han, Protective effects of indole-3-carbinol-loaded poly(lactic-co-glycolic acid) nanoparticles against glutamate-induced neurotoxicity. *J. Nanosci. Nanotechnol.* 15, 7922 **(2015)**.

48. S. F. Rodrigues, L. A. Fiel, A. L. Shimada, N. R. Pereira, S. S. Guterres, A. R. Pohlmann, and S. H. Farsky, Lipid-core nanocapsules act as a drug shuttle through the blood brain barrier and reduce glioblastoma after intravenous or oral administration. *J. Biomed. Nanotechnol.* 12, 986 **(2016)**.

49. K. Hari and P. Kumpati, Chitosan tethered colloidal gold nanospheres for drug delivery applications. *J. Nanosci. Nanotechnol.* 16, 229 **(2016)**.

50. J. Lohr, T. Ratliff, A. Huppertz, Y. Z. Ge, C. Dictus, R. Ahmadi, S. Grau, N. Hiraoka, V. Eckstein, R. C. Ecker, T. Korff, A. von Deimling, A. Unterberg, P. Beckhove, and C. Herold-Mende, Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-beta. *Clin. Cancer Res.* 17, 4296 **(2011)**.

51. G. A. Alexiou, G. Vartholomatos, A. Karamoutsios, A. Batistatou, A. P. Kyritsis, and S. Voulgaris, Circulating progenitor cells: A comparison of patients with glioblastoma or meningioma. *Acta Neurol. Belg.* 113, 7 **(2013)**.

52. Y. C. Ooi, P. Tran, N. Ung, K. Thill, A. Trang, B. M. Fong, D. T. Nagasawa, M. Lim, and I. Yang, The role of regulatory T-cells in glioma immunology. *Clin. Neurol. Neurosur.* 119, 125 **(2014)**.

53. W. Zhou, Z. Jiang, X. G. Li, Y. Y. Xu, and Z. Y. Shao, Cytokines: Shifting the balance between glioma cells and tumor microenvironment after irradiation. *J. Cancer. Res. Clin*. 141, 575 **(2015)**.

54. G. Sciume, A. Santoni, and G. Bernardini, Chemokines and glioma: Invasion and more. *J. Neuroimmunol.* 224, 8 **(2010)**.

55. D. R. Johnson and B. P. O'Neill, Glioblastoma survival in the United States before and during the temozolomide era. *J. Neuro-Oncol.* 107, 359 **(2012)**.

56. E. T. Sayegh, T. Oh, S. Fakurnejad, O. Bloch, and A. T. Parsa, Vaccine therapies for patients with glioblastoma. *J. Neuro-Oncol.* 119, 531 **(2014)**.

57. S. Pessina, G. Cantini, D. Kapetis, E. Cazzato, N. Di Ianni, G. Finocchiaro, and S. Pellegatta, The multidrug-resistance transporter Abcc3 protects NK cells from chemotherapy in a murine model of malignant glioma. *Oncoimmunology* 5 **(2016)**.

58. M. Reni, E. Mazza, S. Zanon, G. Gatta, and C. J. Vecht, Central nervous system gliomas. *Crit. Rev. Oncol. Hemat.* 113, 213 **(2017)**.

59. Y. Liu, Q. Guo, H. Zhang, G. H. Li, S. Feng, X. Z. Yu, L. S. Kong, L. Zhao, and F. Jin, Effect of siRNA-livin on drug resistance to chemotherapy in glioma U251 cells and CD133(+) stem cells. *Exp. Ther. Med.* 10, 1317 **(2015)**.

60. E. Ollier, P. Mazzocco, D. Ricard, G. Kaloshi, A. Idbaih, A. Alentorn, D. Psimaras, J. Honnorat, J. Y. Delattre, E. Grenier, F. Ducray, and A. Samson, Analysis of temozolomide resistance in low-grade gliomas using a mechanistic mathematical model. *Fundam. Clin. Pharmacol.* 31, 347 **(2017)**.

61. R. Mahesparan, T. A. Read, M. Lund-Johansen, K. O. Skaftnesmo, R. Bjerkvig, and O. Engebraaten, Expression of extracellular matrix components in a highly infiltrative *in vivo* glioma model. *Acta Neuropathol.* 105, 49 **(2003)**.

62. H. Di, C. H. Li, X. Y. Wang, C. F. Du, S. P. Sun, Y. D. Li, C. Shang, Q. Wang, and J. H. Wang, Overexpression of miR-874 enhances chemosensitivity of glioma cells to temozolomide by the oncogenic STAT3 pathway. *Int. J. Clin. Exp. Patho.* 10, 2852 **(2017)**.

63. A. Gieryng, D. Pszczolkowska, K. A. Walentynowicz, W. D. Rajan, and B. Kaminska, Immune microenvironment of gliomas. *Lab. Invest.* 97, 498 **(2017)**.

64. D. Schiffer, L. Annovazzi, M. Mazzucco, and M. Mellai, The microenvironment in gliomas: Phenotypic expressions. *Cancers* 7, 2352 **(2015)**.

65. D. F. Quail and J. A. Joyce, The microenvironmental landscape of brain tumors. *Cancer Cell* 31, 326 **(2017)**.

66. J. Chen, L. Lin, Z. P. Guo, C. N. Xu, Y. H. Li, H. Y. Tian, Z. H. Tang, C. L. He, and X. S. Chen, *N*-Isopropylacrylamide modified polyethylenimines as effective siRNA carriers for cancer therapy. *J. Nanosci. Nanotechnol.* 16, 5464 **(2016)**.

67. Y. X. Peng, W. J. Yao, B. Wang, and L. Zong, Mannosylated chitosan nanoparticles based macrophage-targeting gene delivery system enhanced cellular uptake and improved transfection efficiency. *J. Nanosci. Nanotechnol.* 15, 2619 **(2015)**.

68. H. Shen, M. Liu, and Z. J. Zhang, Biodegradable poly(aminoester)-mediated p53 gene delivery for cancer therapy. *J. Nanosci. Nanotechnol.* 16, 2210 **(2016)**.

69. R. Tian, L. Xian, Y. Li, and X. W. Zheng, Silica modified chitosan/polyethylenimine nanogel for improved stability and gene carrier ability. *J. Nanosci. Nanotechnol.* 16, 5426 **(2016)**.

70. G. Z. Qiu, W. Sun, Y. X. Zou, Z. Cai, P. Wang, X. B. Lin, J. X. Huang, L. Jiang, X. H. Ding, and G. H. Hu, RNA interference against TMEM97 inhibits cell proliferation, migration, and invasion in glioma cells. *Tumour Biol.* 36, 8231 **(2015)**.

71. Z. H. Li, Y. Yu, C. Du, H. Fu, J. Wang, and Y. Tian, RNA interference-mediated USP22 gene silencing promotes human brain

glioma apoptosis and induces cell cycle arrest. *Oncol. Lett.* 5, 1290 **(2013)**.
72. Q. W. Fan and W. A. Weiss, RNA interference against a glioma-derived allele of EGFR induces blockade at G2M. *Oncogene* 24, 829 **(2005)**.
73. A. Fire, S. Q. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello, Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 391, 806 **(1998)**.
74. M. T. McManus and P. A. Sharp, Gene silencing in mammals by small interfering RNAs. *Nat. Rev. Genet.* 3, 737 **(2002)**.
75. N. A. Charles, E. C. Holland, R. Gilbertson, R. Glass, and H. Kettenmann, The brain tumor microenvironment. *Glia* 59, 1169 **(2011)**.
76. D. F. Quail, R. L. Bowman, L. Akkari, M. L. Quick, A. J. Schuhmacher, J. T. Huse, E. C. Holland, J. C. Sutton, and J. A. Joyce, The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. *Science* 352, aad3018 **(2016)**.
77. J. Qiao, M. Dey, A. L. Chang, J. W. Kim, J. Miska, A. Ling, D. M. Nettlebeck, Y. Han, L. J. Zhang, and M. S. Lesniak, Intratumoral oncolytic adenoviral treatment modulates the glioma microenvironment and facilitates systemic tumor-antigen-specific T cell therapy. *Oncoimmunology* 4 **(2015)**.
78. T. L. Whiteside, The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 27, 5904 **(2008)**.
79. J. A. Joyce and J. W. Pollard, Microenvironmental regulation of metastasis. *Nat. Rev. Cancer* 9, 239 **(2009)**.
80. Q. Y. Hu, W. J. Sun, Y. Lu, H. N. Bomba, Y. Q. Ye, T. Y. Jiang, A. J. Isaacson, and Z. Gu, Tumor microenvironment-mediated construction and deconstruction of extracellular drug-delivery depots. *Nano Lett.* 16, 1118 **(2016)**.
81. T. Wu and Y. Dai, Tumor microenvironment and therapeutic response. *Cancer Lett.* 387, 61 **(2017)**.
82. D. H. Gutmann, Microglia in the tumor microenvironment: Taking their TOLL on glioma biology. *Neuro-Oncol.* 17, 171 **(2015)**.
83. K. K. Goswami, T. Ghosh, S. Ghosh, M. Sarkar, A. Bose, and R. Baral, Tumor promoting role of anti-tumor macrophages in tumor microenvironment. *Cell Immunol.* 316, 1 **(2017)**.
84. C. X. Hu and X. D. Jiang, The effect of anti-angiogenic drugs on regulatory T cells in the tumor microenvironment. *Biomed. Pharmacother.* 88, 134 **(2017)**.
85. C. Pfirschke, M. Siwicki, H. W. Liao, and M. J. Pittet, Tumor microenvironment: No effector T cells without dendritic cells. *Cancer Cell* 31, 614 **(2017)**.
86. M. H. Kim and H. J. Jeong, Zinc oxide nanoparticles suppress LPS-induced NF-kappaB activation by inducing A20, a negative regulator of NF-kappaB, in RAW 264.7 macrophages. *J. Nanosci. Nanotechnol.* 15, 6509 **(2015)**.
87. W. H. Lee, W. E. Sonntag, and Y. W. Lee, Aging attenuates radiation-induced expression of pro-inflammatory mediators in rat brain. *Neuroscience Lett.* 476, 89 **(2010)**.
88. W. H. Lee, W. E. Sonntag, M. Mitschelen, H. Yan, and Y. W. Lee, Irradiation induces regionally specific alterations in pro-inflammatory environments in rat brain. *Int. J. Radiat. Biol.* 86, 132 **(2010)**.
89. X. R. Cui, J. P. Liu, L. Bai, J. Tian, and J. Zhu, Interleukin-6 induces malignant transformation of rat mesenchymal stem cells in association with enhanced signaling of signal transducer and activator of transcription 3. *Cancer Sci.* 105, 64 **(2014)**.
90. K. Motomura, A. Natsume, Y. Kishida, H. Higashi, Y. Kondo, Y. Nakasu, T. Abe, H. Namba, K. Wakai, and T. Wakabayashi, Benefits of interferon-β and temozolomide combination therapy for newly diagnosed primary glioblastoma with the unmethylated MGMT promoter: A multicenter study. *Cancer* 117, 1721 **(2011)**.
91. B. R. Persson, C. B. Koch, G. Grafstrom, C. Ceberg, P. M. Rosenschold, H. Nittby, B. Widegren, and L. G. Salford, Radiation immunomodulatory gene tumor therapy of rats with intracerebral glioma tumors. *Radiat. Res.* 173, 433 **(2010)**.
92. U. Demirci, M. Yaman, S. Buyukberber, U. Coskun, M. Baykara, K. Uslu, A. Ozet, M. Benekli, and E. U. Bagriacik, Prognostic importance of markers for inflammation, angiogenesis and apoptosis in high grade glial tumors during temozolomide and radiotherapy. *Int. Immunopharmacol.* 14, 546 **(2012)**.
93. J. R. Pineda, M. Daynac, A. Chicheportiche, A. Cebrian-Silla, K. Sii Felice, J. M. Garcia-Verdugo, F. D. Boussin, and M. A. Mouthon, Vascular-derived TGF-beta increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. *EMBO Mol. Med.* 5, 548 **(2013)**.
94. B. Raychaudhuri and M. A. Vogelbaum, IL-8 is a mediator of NF-kappa B induced invasion by gliomas. *J. Neuro-Oncol.* 101, 227 **(2011)**.
95. S. Rao, R. Sengupta, E. J. Choe, B. M. Woerner, E. Jackson, T. Sun, J. Leonard, D. Piwnica-Worms, and J. B. Rubin, CXCL12 mediates trophic interactions between endothelial and tumor cells in glioblastoma. *PloS One* 7, e33005 **(2012)**.
96. A. Zisakis, C. Piperi, M. S. Themistocleous, P. Korkolopoulou, E. I. Boviatsis, D. E. Sakas, E. Patsouris, R. W. Lea, and A. Kalofoutis, Comparative analysis of peripheral and localised cytokine secretion in glioblastoma patients. *Cytokine* 39, 99 **(2007)**.
97. D. H. Munn and V. Bronte, Immune suppressive mechanisms in the tumor microenvironment. *Curr. Opin. Immunol.* 39, 1 **(2016)**.
98. Y. Liu and X. T. Cao, Immunosuppressive cells in tumor immune escape and metastasis. *J. Mol. Med.* 94, 509 **(2016)**.
99. P. Perng and M. Lim, Immunosuppressive mechanisms of malignant gliomas: Parallels at non-CNS sites. *Front. Oncol.* 5, 153 **(2015)**.
100. C. S. Kang, P. Y. Pu, Y. H. Li, Z. Y. Zhang, M. Z. Qiu, Q. Huang, and G. X. Wang, An *in vitro* study on the suppressive effect of glioma cell growth induced by plasmid-based small interference RNA (siRNA) targeting human epidermal growth factor receptor. *J. Neuro-Oncol.* 74, 267 **(2005)**.
101. V. Umesh, A. D. Rape, T. A. Ulrich, and S. Kumar, Microenvironmental stiffness enhances glioma cell proliferation by stimulating epidermal growth factor receptor signaling. *PloS One* 9, e101771 **(2014)**.
102. A. H. Liao, H. Y. Chou, Y. L. Hsieh, S. C. Hsu, K. C. Wei, and H. L. Liu, Enhanced therapeutic epidermal growth factor receptor (EGFR) antibody delivery via pulsed ultrasound with targeting microbubbles for glioma treatment. *J. Med. Biol. Eng.* 35, 156 **(2015)**.
103. A. P. See, J. E. Han, J. Phallen, Z. Binder, G. Gallia, F. Pan, D. Jinasena, C. Jackson, Z. Belcaid, S. J. Jeong, C. Gottschalk, J. Zeng, J. Ruzevick, S. Nicholas, Y. Kim, E. Albesiano, D. M. Pardoll, and M. Lim, The role of STAT3 activation in modulating the immune microenvironment of GBM. *J. Neuro-Oncol.* 110, 359 **(2012)**.
104. J. Mieczkowski, M. Kocyk, P. Nauman, K. Gabrusiewicz, M. Sielska, P. Przanowski, M. Maleszewska, W. D. Rajan, D. Pszczolkowska, T. Tykocki, W. Grajkowska, K. Kotulska, M. Roszkowski, B. Kostkiewicz, and B. Kaminska, Down-regulation of IKK beta expression in glioma-infiltrating microglia/macrophages is associated with defective inflammatory/immune gene responses in glioblastoma. *Oncotarget* 6, 33077 **(2015)**.
105. E. Turano, A. Farinazzo, E. Bistaffa, B. Bonetti, and E. Bazzoli, Role of nanovesicles from macrophages/microglia in the cross-talk between glioma cells and microenvironment. *J. Neuroimmunol.* 275, 40 **(2014)**.
106. J. Wei, K. Gabrusiewicz, and A. Heimberger, The Controversial Role of Microglia in Malignant Gliomas. *Clin. Dev. Immunol.* 2013, 285246 **(2013)**.
107. J. R. Engler, A. E. Robinson, I. Smirnov, J. G. Hodgson, M. S. Berger, N. Gupta, C. D. James, A. Molinaro, and J. J. Phillips, Increased microglia/macrophage gene expression in a subset of adult and pediatric astrocytomas. *PloS One* 7, e43339 **(2012)**.

108. H. Ogbomo, J. Cinatl, C. H. Mody, and P. A. Forsyth, Immunotherapy in gliomas: Limitations and potential of natural killer (NK) cell therapy. *Trends. Mol. Med.* 17, 433 **(2011)**.

109. C. Jackson, J. Ruzevick, J. Phallen, Z. Belcaid, and M. Lim, Challenges in immunotherapy presented by the glioblastoma multiforme microenvironment. *Clin. Dev. Immunol.* 2011, 1 **(2011)**.

110. B. Y. Huang, Y. P. Zhan, W. J. Zong, C. J. Yu, J. F. Li, Y. M. Qu, and S. Han, The PD-1/B7-H1 pathway modulates the natural killer cells versus mouse glioma stem cells. *PloS One* 10, e0134715 **(2015)**.

111. H. Okada, G. Kohanbash, X. M. Zhu, E. R. Kastenhuber, A. Hoji, R. Ueda, and M. Fujita, Immunotherapeutic approaches for glioma. *Crit. Rev. Immunol.* 29, 1 **(2009)**.

112. A. Authier, K. J. Farrand, K. W. R. Broadley, L. R. Ancelet, M. K. Hunn, S. Stone, M. J. McConnell, and I. F. Hermans, Enhanced immunosuppression by therapy-exposed glioblastoma multiforme tumor cells. *Int. J. Cancer* 136, 2566 **(2015)**.

113. L. M. Phillips, X. H. Zhou, D. E. Cogdell, C. Y. Chua, A. Huisinga, K. R. Hess, G. N. Fuller, and W. Zhang, Glioma progression is mediated by an addiction to aberrant IGFBP2 expression and can be blocked using anti-IGFBP2 strategies. *J. Pathol.* 239, 355 **(2016)**.

114. S. Z. Lv, B. W. Sun, C. X. Dai, R. R. Shi, X. T. Zhou, W. Y. Lv, X. Zhong, R. Z. Wang, and W. B. Ma, The downregulation of microRNA-146a modulates TGF-beta signaling pathways activity in glioblastoma. *Mol. Neurobiol.* 52, 1257 **(2015)**.

115. S. Z. Lv, J. Qin, R. Y. Yi, M. Coreman, R. R. Shi, H. H. Kang, and C. J. Yao, CrkL efficiently mediates cell proliferation, migration, and invasion induced by TGF-β pathway in glioblastoma. *J. Mol. Neurosci.* 51, 1046 **(2013)**.

116. T. Tsuchida, R. E. Saxton, and R. F. Irie, Gangliosides of human melanoma: GM2 and tumorigenicity. *J. Natl. Cancer I* 78, 55 **(1987)**.

117. O. Nakamura, M. Iwamori, M. Matsutani, and K. Takakura, Ganglioside Gd3 shedding by human gliomas. *Acta Neurochir.* 109, 34 **(1991)**.

118. R. Wagener, G. Rohn, G. Schillinger, R. Schroder, B. Kobbe, and R. I. Ernestus, Ganglioside profiles in human gliomas: Quantification by microbore high performance liquid chromatography and correlation to histomorphology and grading. *Acta Neurochir.* 141, 1339 **(1999)**.

119. G. Sa, T. Das, C. Moon, C. M. Hilston, P. A. Rayman, B. I. Rini, C. S. Tannenbaum, and J. H. Finke, GD3, an overexpressed tumor-derived ganglioside, mediates the apoptosis of activated but not resting T cells. *Cancer Res.* 69, 3095 **(2009)**.

120. B. Mahata, S. Biswas, P. Rayman, A. Chahlavi, J. Ko, A. Bhattacharjee, Y. T. Li, Y. T. Li, T. Das, G. Sa, B. Raychaudhuri, M. A. Vogelbaum, C. Tannenbaum, J. H. Finke, and K. Biswas, GBM derived gangliosides induce T cell apoptosis through activation of the caspase cascade involving both the extrinsic and the intrinsic pathway. *PloS One* 10, e0134425 **(2015)**.

121. L. Y. Zhang, M. Van Handel, J. M. Schartner, A. Hagar, G. Allen, M. Curet, and B. Badie, Regulation of IL-10 expression by upstream stimulating factor (USF-1) in glioma-associated microglia. *J. Neuroimmunol.* 184, 188 **(2007)**.

122. S. De Vleeschouwer, I. S. Lopes, J. L. Ceuppens, and S. W. Van Gool, Persistent IL-10 production is required for glioma growth suppressive activity by Th1-directed effector cells after stimulation with tumor lysate-loaded dendritic cells. *J. Neuro-Oncol.* 84, 131 **(2007)**.

123. B. Qiu, D. Y. Zhang, C. Wang, J. Tao, X. X. Tie, Y. Qiao, K. Xu, Y. J. Wang, and A. H. Wu, IL-10 and TGF-beta 2 are overexpressed in tumor spheres cultured from human gliomas. *Mol. Biol. Rep.* 38, 3585 **(2011)**.

124. W. T. Couldwell, P. Doreduffy, M. L. J. Apuzzo, and J. P. Antel, Malignant glioma modulation of immune function: Relative contribution of different soluble factors. *J. Neuroimmunol.* 33, 89 **(1991)**.

125. G. M. Lauro, N. Dilorenzo, M. Grossi, A. Maleci, and B. Guidetti, Prostaglandin-E2 as an immunomodulating factor released *in vitro* by human glioma-cells. *Acta Neuropathol.* 69, 278 **(1986)**.

126. Y. Sawamura, A. C. Diserens, and N. de Tribolet, *In vitro* prostaglandin E2 production by glioblastoma cells and its effect on interleukin-2 activation of oncolytic lymphocytes. *J. Neuro-Oncol.* 9, 125 **(1990)**.

127. L. Lalier, P. F. Cartron, F. Pedelaborde, C. Olivier, D. Loussouarn, S. A. Martin, K. Meflah, J. Menanteau, and F. M. Vallette, Increase in PGE2 biosynthesis induces a Bax dependent apoptosis correlated to patients' survival in glioblastoma multiforme. *Oncogene* 26, 4999 **(2007)**.

128. H. Okada, L. Villa, J. Attanucci, M. Erff, W. K. Fellows, M. T. Lotze, I. F. Pollack, and W. H. Chambers, Cytokine gene therapy of gliomas: Effective induction of therapeutic immunity to intracranial tumors by peripheral immunization with interleukin-4 transduced glioma cells. *Gene Ther.* 8, 1157 **(2001)**.

129. S. Fukui, H. Nawashiro, N. Otani, H. Ooigawa, N. Nomura, A. Yano, T. Miyazawa, A. Ohnuki, N. Tsuzuki, H. Katoh, S. Ishihara, and K. Shima, Nuclear accumulation of basic fibroblast growth factor in human astrocytic tumors—A poor prognostic factor. *Cancer* 97, 3061 **(2003)**.

130. B. Zhang, X. Q. Feng, J. H. Wang, X. N. Xu, N. Lin, and H. S. Liu, Combined antitumor effect of Ad-bFGF-siRNA and Ad-Vpr on the growth of xenograft glioma in nude mouse model. *Pathol. Oncol. Res.* 17, 237 **(2011)**.

131. L. Tarassishin, D. Casper, and S. C. Lee, Aberrant expression of interleukin-1 beta and inflammasome activation in human malignant gliomas. *PloS One* 9, e103432 **(2014)**.

132. B. X. Wang, R. Rahbar, and E. N. Fish, Interferon: Current status and future prospects in cancer therapy. *J. Interf. Cytok. Res.* 31, 545 **(2011)**.

133. M. Pickup, S. Novitskiy, and H. L. Moses, The roles of TGF beta in the tumour microenvironment. *Nat. Rev. Cancer* 13, 788 **(2013)**.

134. L. O. Roy, M. B. Poirier, and D. Fortin, Transforming growth factor-beta and its implication in the malignancy of gliomas. *Target Oncol.* 10, 1 **(2015)**.

135. J. F. Han, C. A. Alvarez-Breckenridge, Q. E. Wang, and J. H. Yu, TGF-beta signaling and its targeting for glioma treatment. *Am. J. Cancer Res.* 5, 945 **(2015)**.

136. M. H. Yazdi, B. Varastehmoradi, E. Faghfuri, F. Mavandadnejad, M. Mandavi, and A. R. Shahverdi, Adjuvant effect of biogenic selenium nanoparticles improves the immune responses and survival of mice receiving 4T1 cell antigens as vaccine in breast cancer murine model. *J. Nanosci. Nanotechnol.* 15, 10165 **(2015)**.

137. J. Sun, S. Z. Liu, Y. Lin, X. P. Cao, and J. M. Liu, TGF-beta promotes glioma cell growth via activating nodal expression through smad and ERK1/2 pathways. *Biochem. Bioph. Res. Co.* 443, 1066 **(2014)**.

138. X. J. Yang, G. L. Chen, S. C. Yu, C. Xu, Y. H. Xin, T. T. Li, Y. Shi, A. Gu, J. J. Duan, C. Qian, Y. H. Cui, X. Zhang, and X. W. Bian, TGF-beta 1 enhances tumor-induced angiogenesis via JNK pathway and macrophage infiltration in an improved zebrafish embryo/xenograft glioma model. *Int. Immunopharmacol.* 15, 191 **(2013)**.

139. Y. Lu, F. Jiang, X. G. Zheng, M. Katakowski, B. Buller, S. S. T. To, and M. Chopp, TGF-beta 1 promotes motility and invasiveness of glioma cells through activation of ADAM17. *Oncol. Rep.* 25, 1329 **(2011)**.

140. M. A. Friese, J. Wischhusen, W. Wick, M. Weiler, G. Eisele, A. Steinle, and M. Weller, RNA interference targeting transforming growth factor-beta enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity *in vivo*. *Cancer Res.* 64, 7596 **(2004)**.

141. C. Seliger, P. Leukel, S. Moeckel, B. Jachnik, C. Lottaz, M. Kreutz, A. Brawanski, M. Proescholdt, U. Bogdahn, A. K. Bosserhoff,

A. Vollmann-Zwerenz, and P. Hau, Lactate-modulated induction of THBS-1 activates transforming growth factor (TGF)-beta 2 and migration of glioma cells *in vitro*. *PloS One* 8, 70 **(2013)**.

142. D. N. Irani, K. I. Lin, and D. E. Griffin, Brain-derived gangliosides regulate the cytokine production and proliferation of activated T cells. *J. Immunol.* 157, 4333 **(1996)**.

143. E. Albesiano, J. E. Han, and M. Lim, Mechanisms of local immunoresistance in glioma. *Neurosurg. Clin. of N. Am.* 21, 17 **(2010)**.

144. C. Meyer, G. Martin-Blondel, and R. S. Liblau, Endothelial cells and lymphatics at the interface between the immune and central nervous systems: implications for multiple sclerosis. *Curr. Opin. Neurol.* 30, 222 **(2017)**.

145. T. Suter, G. Biollaz, D. Gatto, L. Bernasconi, T. Herren, W. Reith, and A. Fontana, The brain as an immune privileged site: Dendritic cells of the central nervous system inhibit T cell activation. *Eur J. Immunol.* 33, 2998 **(2003)**.

146. B. Engelhardt, P. Vajkoczy, and R. O. Weller, The movers and shapers in immune privilege of the CNS. *Nat. Immunol.* 18, 123 **(2017)**.

147. D. Fortuna, A. M. Cardenas, E. H. Graf, L. A. Harshyne, D. C. Hooper, M. Prosniak, J. Shields, and M. T. Curtis, Human parechovirus and enterovirus initiate distinct CNS innate immune responses: Pathogenic and diagnostic implications. *J. Clin. Virol.* 86, 39 **(2017)**.

148. S. Brandenburg, K. Turkowski, A. Mueller, Y. T. Radev, S. Seidlitz, and P. Vajkoczy, Myeloid cells expressing high level of CD45 are associated with a distinct activated phenotype in glioma. *Immunol. Res.* 65, 757 **(2017)**.

149. B. Ahn, G. Kohanbash, T. Ohkuri, A. Kosaka, X. Chen, M. Ikeura, T. C. Wang, and H. Okada, Histamine deficiency promotes accumulation of immunosuppressive immature myeloid cells and growth of murine gliomas. *Oncoimmunology* 4, e1047581 **(2015)**.

150. B. Raychaudhuri, P. Rayman, P. J. Huang, M. Grabowski, D. Hambardzumyan, J. H. Finke, and M. A. Vogelbaum, Myeloid derived suppressor cell infiltration of murine and human gliomas is associated with reduction of tumor infiltrating lymphocytes. *J. Neuro-Oncol.* 122, 293 **(2015)**.

151. R. Glass and M. Synowitz, CNS macrophages and peripheral myeloid cells in brain tumours. *Acta Neuropathol.* 128, 347 **(2014)**.

152. W. Hua, Y. Yao, Y. W. Chu, P. Zhong, X. F. Sheng, B. G. Xiao, J. S. Wu, B. J. Yang, Y. Mao, and L. F. Zhou, The CD133+ tumor stem-like cell-associated antigen may elicit highly intense immune responses against human malignant glioma. *J. Neuro-Oncol.* 105, 149 **(2011)**.

153. L. S. Ge, A. N. Cornforth, N. T. Hoa, C. Delgado, S. K. Chiou, Y. H. Zhou, and M. R. Jadus, Differential glioma-associated tumor antigen expression profiles of human glioma cells grown in hypoxia. *PloS One* 7, e42661 **(2012)**.

154. K. Saito, S. Ohta, Y. Kawakami, K. Yoshida, and M. Toda, Functional analysis of KIF20A, a potential immunotherapeutic target for glioma. *J. Neuro-Oncol.* 132, 63 **(2017)**.

155. I. F. Pollack, R. I. Jakacki, L. H. Butterfield, R. L. Hamilton, A. Panigrahy, D. P. Normolle, A. K. Connelly, S. Dibridge, G. Mason, T. L. Whiteside, and H. Okada, Antigen-specific immunoreactivity and clinical outcome following vaccination with glioma-associated antigen peptides in children with recurrent high-grade gliomas: Results of a pilot study. *J. Neuro-Oncol.* 130, 517 **(2016)**.

156. I. Yang, S. J. Han, G. Kaur, C. Crane, and A. T. Parsa, The role of microglia in central nervous system immunity and glioma immunology. *J. Clin. Neurosci.* 17, 6 **(2010)**.

157. T. Masuda and M. Prinz, Microglia: A unique versatile cell in the central nervous system. *Acs. Chem. Neurosci.* 7, 428 **(2016)**.

158. D. P. Schafer and B. Stevens, Microglia function in central nervous system development and plasticity. *Csh. Perspect. Biol.* 7 **(2015)**.

159. G. Kaur, S. J. Han, I. Yang, and C. Crane, Microglia and central nervous system immunity. *Neurosurg. Clin. N. Am.* 21, 43 **(2010)**.

160. T. E. Peterson, N. D. Kirkpatrick, Y. H. Huang, C. T. Farrar, K. A. Marijt, J. Kloepper, M. Datta, Z. Amoozgar, G. Seano, K. Jung, W. S. Kamoun, T. Vardam, M. Snuderl, J. Goveia, S. Chatterjee, A. Batista, A. Muzikansky, C. C. Leow, L. Xu, T. T. Batchelor, D. G. Duda, D. Fukumura, and R. K. Jain, Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. *P. Natl. Acad. Sci. USA* 113, 4470 **(2016)**.

161. R. Franco and D. Fernandez-Suarez, Alternatively activated microglia and macrophages in the central nervous system. *Prog. Neurobiol.* 131, 65 **(2015)**.

162. D. Alizadeh, L. Y. Zhang, J. Hwang, T. Schluep, and B. Badie, Tumor-associated macrophages are predominant carriers of cyclodextrin-based nanoparticles into gliomas. *Nanomed-Nanotechnol.* 6, 382 **(2010)**.

163. L. Y. Zhang, D. Alizadeh, M. Van Handel, M. Kortylewski, H. Yu, and B. Badie, Stat3 inhibition activates tumor macrophages and abrogates glioma growth in mice. *Glia* 57, 1458 **(2009)**.

164. R. Wang, J. L. Zhang, B. Wei, Y. Tian, Z. H. Li, L. Wang, and C. Du, Upregulation of plasmacytoid dendritic cells in glioma. *Tumor Biol.* 35, 9661 **(2014)**.

165. H. Assi, J. Espinosa, S. Suprise, M. Sofroniew, R. Doherty, D. Zamler, P. R. Lowenstein, and M. G. Castro, Assessing the Role of STAT3 in DC differentiation and autologous DC immunotherapy in mouse models of GBM. *PloS One* 9, e96318 **(2014)**.

166. T. V. Tyrinova, O. Y. Leplina, S. V. Mishinov, M. A. Tikhonova, E. Y. Shevela, V. V. Stupak, I. V. Pendyurin, A. G. Shilov, E. A. Alyamkina, N. V. Rubtsova, S. S. Bogachev, A. A. Ostanin, and E. R. Chernykh, Cytotoxic activity of *ex-vivo* generated IFN alpha-induced monocyte-derived dendritic cells in brain glioma patients. *Cell Immunol.* 284, 146 **(2013)**.

167. E. A. Ling, Y. K. Ng, C. H. Wu, and C. Kaur, Microglia: Its development and role as a neuropathology sensor. *Prog. Brain Res.* 132, 61 **(2001)**.

168. T. Kees, J. Lohr, J. Noack, R. Mora, G. Gdynia, G. Todt, A. Ernst, B. Radlwimmer, C. S. Falk, C. Herold-Mende, and A. Regnier-Vigouroux, Microglia isolated from patients with glioma gain antitumor activities on poly (I:C) stimulation. *Neuro-Oncol.* 14, 64 **(2012)**.

169. S. F. Hussain, D. Yang, D. Suki, K. Aldape, E. Grimm, and A. B. Heimberger, The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. *Neuro-Oncol.* 8, 261 **(2006)**.

170. J. T. Miyauchi, D. L. Chen, M. Choi, J. C. Nissen, K. R. Shroyer, S. Djordevic, I. C. Zachary, D. Selwood, and S. E. Tsirka, Ablation of Neuropilin 1 from glioma-associated microglia and macrophages slows tumor progression. *Oncotarget* 7, 9801 **(2016)**.

171. A. Wesolowska, A. Kwiatkowska, L. Slomnicki, M. Dembinski, A. Master, M. Sliwa, K. Franciszkiewicz, S. Chouaib, and B. Kaminska, Microglia-derived TGF-beta as an important regulator of glioblastoma invasion—An inhibition of TGF-beta-dependent effects by shRNA against human TGF-beta type II receptor. *Oncogene* 27, 918 **(2008)**.

172. A. Ghoochani, M. A. Schwarz, E. Yakubov, T. Engelhorn, A. Doerfler, M. Buchfelder, R. Bucala, N. E. Savaskan, and I. Y. Eyupoglu, MIF-CD74 signaling impedes microglial M1 polarization and facilitates brain tumorigenesis. *Oncogene 35*, 1 **(2016)**.

173. C. H. Jones, M. F. Chen, A. Ravikrishnan, R. Reddinger, G. J. Zhang, A. P. Hakansson, and B. A. Pfeifer, Mannosylated poly(beta-amino esters) for targeted antigen presenting cell immune modulation. *Biomaterials* 37, 333 **(2015)**.

174. M. Mohammad, V. W. W. Tsai, M. Ruitenberg, M. Hassanpour, H. Li, P. Hart, S. Breit, P. Sawchenko, and D. Brown, CNS DCs modulate peripheral anti-CNS immunity. *J. Neuroimmunol.* 275, 82 **(2014)**.

175. Y. Y. Huang, Q. Q. Mao, J. He, J. Su, Y. Peng, W. Liang, Z. X. Hu, S. F. Zhou, X. L. Lu, and Y. X. Zhao, Fusions of tumor-derived endothelial cells with dendritic cells induces antitumor immunity. *Sci. Rep.* 7 **(2017)**.

176. I. Mellman and R. M. Steinman, Dendritic cells: Specialized and regulated antigen processing machines. *Cell* 106, 255 **(2001)**.

177. J. J. Engelhardt, B. Boldajipour, P. Beemiller, P. Pandurangi, C. Sorensen, Z. Werb, M. Egeblad, and M. F. Krummel, Marginating dendritic cells of the tumor microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. *Cancer Cell* 21, 402 **(2012)**.

178. B. Platzer, K. G. Elpek, V. Cremasco, K. Baker, M. M. Stout, C. Schultz, E. Dehlink, K. T. C. Shade, R. M. Anthony, R. S. Blumberg, S. J. Turley, and E. Fiebiger, IgE/Fc epsilon RI-mediated antigen cross-presentation by dendritic cells enhances anti-tumor immune responses. *Cell Rep.* 10, 1487 **(2015)**.

179. A. M. McDonnell, W. J. Lesterhuis, A. Khong, A. K. Nowak, R. A. Lake, A. J. Currie, and B. W. S. Robinson, Tumor-infiltrating dendritic cells exhibit defective cross-presentation of tumor antigens, but is reversed by chemotherapy. *Eur. J. Immunol.* 45, 49 **(2015)**.

180. C. Figueroa, F. Galvez-Cancino, C. Oyarce, F. Contreras, C. Prado, C. Valeria, S. Cruz, A. Lladser, and R. Pacheco, Inhibition of dopamine receptor D3 signaling in dendritic cells increases antigen cross-presentation to CD8(+) T-cells favoring anti-tumor immunity. *J. Neuroimmunol.* 303, 99 **(2017)**.

181. N. Kibbi, O. Sobolev, M. Girardi, and R. L. Edelson, Induction of anti-tumor CD8 T cell responses by experimental ECP-induced human dendritic antigen presenting cells. *Transfus. Apher. Sci.* 55, 146 **(2016)**.

182. M. T. Vitor, P. C. Bergami-Santos, K. S. P. Cruz, M. P. Pinho, J. A. M. Barbuto, and L. G. De La Torre, Dendritic cells stimulated by cationic liposomes. *J. Nanosci. Nanotechnol.* 16, 270 **(2016)**.

183. C. A. Janeway and K. Bottomly, Signals and signs for lymphocyte-responses. *Cell* 76, 275 **(1994)**.

184. M. Hansen and M. H. Andersen, The role of dendritic cells in cancer. *Semin. Immunopathol.* 39, 307 **(2017)**.

185. S. Y. Chang, J. H. Song, B. Guleng, C. A. Cotoner, S. Arihiro, Y. Zhao, H. S. Chiang, M. O'Keeffe, G. X. Liao, C. L. Karp, M. N. Kweon, A. H. Sharpe, A. Bhan, C. Terhorst, and H. C. Reinecker, Circulatory antigen processing by mucosal dendritic cells controls CD8(+) T cell activation. *Immunity* 38, 153 **(2013)**.

186. D. Dudziak, A. O. Kamphorst, G. F. Heidkamp, V. R. Buchholz, C. Trumpfheller, S. Yamazaki, C. Cheong, K. Liu, H. W. Lee, C. G. Park, R. M. Steinman, and M. C. Nussenzweig, Differential antigen processing by dendritic cell subsets *in vivo*. *Science* 315, 107 **(2007)**.

187. J. H. Kim, T. H. Kang, K. H. Noh, S. H. Kim, Y. H. Lee, K. W. Kim, H. C. Bae, Y. H. Ahn, E. Y. Choi, J. S. Kim, K. M. Lee, and T. W. Kim, Enhancement of DC vaccine potency by activating the PI3K/AKT pathway with a small interfering RNA targeting PTEN. *Immunol. Lett.* 134, 47 **(2010)**.

188. G. T. Liu, H. Ng, Y. Akasaki, X. P. Yuan, M. Ehtesham, D. Yin, K. L. Black, and J. S. Yu, Small interference RNA modulation of IL-10 in human monocyte-derived dendritic cells enhances the Th1 response. *Eur. J. Immunol.* 34, 1680 **(2004)**.

189. Y. H. Ahn, S. O. Hong, J. H. Kim, K. H. Noh, K. H. Song, Y. H. Lee, J. H. Jeon, D. W. Kim, J. H. Seo, and T. W. Kim, The siRNA cocktail targeting interleukin 10 receptor and transforming growth factor-receptor on dendritic cells potentiates tumour antigen-specific CD8(+) T cell immunity. *Clin. Exp. Immunol.* 181, 164 **(2015)**.

190. C. C. G. Naus, J. F. Bechberger, S. Caveney, and J. X. Wilson, Expression of gap junction genes in astrocytes and C6 glioma-cells. *Neurosci. Lett.* 126, 33 **(1991)**.

191. X. T. Hong, W. C. Sin, A. L. Harris, and C. C. Naus, Gap junctions modulate glioma invasion by direct transfer of microRNA. *Oncotarget* 6, 15566 **(2015)**.

192. W. C. Sin, J. F. Bechberger, W. J. Rushlow, and C. C. Naus, Dose-dependent differential upregulation of CCN1/Cyr61 and CCN3/NOV by the gap junction protein connexin43 in glioma cells. *J. Cell Biochem.* 103, 1772 **(2008)**.

193. A. Haghikia, K. Ladage, P. Lafenetre, A. Haghikia, D. Hinkerohe, D. Smikalla, C. G. Haase, R. Dermietzel, and P. M. Faustmann, Intracellular application of TNF-alpha impairs cell to cell communication via gap junctions in glioma cells. *J. Neuro-Oncol.* 86, 143 **(2008)**.

194. L. Soroceanu, T. J. Manning, and H. Sontheimer, Reduced expression of connexin-43 and functional gap junction coupling in human gliomas. *Glia* 33, 107 **(2001)**.

195. W. L. Chen, D. H. Wang, X. W. Du, Y. He, S. Y. Chen, Q. Q. Shao, C. Ma, B. Huang, A. J. Chen, P. Zhao, X. Qu, and X. G. Li, Glioma cells escaped from cytotoxicity of temozolomide and vincristine by communicating with human astrocytes. *Med. Oncol.* 32, 43 **(2015)**.

196. W. Zhang, W. T. Couldwell, M. F. Simard, H. Song, J. H. C. Lin, and M. Nedergaard, Direct gap junction communication between malignant glioma cells and astrocytes. *Cancer Res.* 59, 1994 **(1999)**.

197. W. C. Sin, Q. Aftab, J. F. Bechberger, J. H. Leung, H. Chen, and C. C. Naus, Astrocytes promote glioma invasion via the gap junction protein connexin43. *Oncogene* 35, 1504 **(2016)**.

198. R. Oliveira, C. Christov, J. S. Guillamo, S. DeBouard, S. Palfi, L. Venance, M. Tardy, and M. Peschanski, Contribution of gap junctional communication between tumor cells and astroglia to the invasion of the brain parenchyma by human glioblastomas. *Bmc. Cell. Biol.* 6, 7 **(2005)**.

199. S. F. Ziegler, Division of Labour by Cd4(+) T Helper Cells. *Nat. Rev. Immunol.* 16, 403 **(2016)**.

200. I. Puliaeva, R. Puliaev, and C. S. Via, Therapeutic potential of CD8+cytotoxic T lymphocytes in SLE. *Autoimmun. Rev.* 8, 219 **(2009)**.

201. A. Tanaka and S. Sakaguchi, Regulatory T cells in cancer immunotherapy. *Cell Res.* 27, 109 **(2017)**.

202. B. Khor, Regulatory T cells: Central concepts from ontogeny to therapy. *Transfus. Med. Rev.* 31, 36 **(2017)**.

203. R. Lin, L. Chen, G. Chen, C. Y. Hu, S. Jiang, J. Sevilla, Y. Wan, J. H. Sampson, B. Zhu, and Q. J. Li, Targeting miR-23a in CD8(+) cytotoxic T lymphocytes prevents tumor-dependent immunosuppression. *J. Clin. Invest.* 124, 5352 **(2014)**.

204. R. Amaraa, H. Mareckova, P. Urbanek, and T. Fucikova, T helper, cytotoxic T lymphocyte, NK cell and NK-T cell subpopulations in patients with chronic hepatitis C. *Folia Microbiol.* 47, 717 **(2002)**.

205. W. Maes, T. Verschuere, A. Van Hoylandt, L. Boon, and S. Van Gool, Depletion of regulatory T cells in a mouse experimental glioma model through Anti-CD25 treatment results in the infiltration of non-immunosuppressive myeloid cells in the Brain. *Clin. Dev. Immunology* 2013, 952469 **(2013)**.

206. A. E. Carpentier and Y. Meng, Recent advances in immunotherapy for human glioma. *Curr. Opin. Oncol.* 18, 631 **(2006)**.

207. I. F. Parney, Basic concepts in glioma immunology. *Glioma: Immunotherapeutic Approaches* 746, 42 **(2012)**.

208. S. Sengupta, S. Nandi, E. S. Hindi, D. A. Wainwright, Y. Han, and M. S. Lesniak, Short hairpin RNA-mediated fibronectin knockdown delays tumor growth in a mouse glioma model. *Neoplasia* 12, 837 **(2010)**.

209. T. Verschuere, J. Toelen, W. Maes, F. Poirier, L. Boon, T. Tousseyn, T. Mathivet, H. Gerhardt, V. Mathieu, R. Kiss, F. Lefranc, S. W. Van Gool, and S. De Vleeschouwer, Glioma-derived galectin-1 regulates innate and adaptive antitumor immunity. *Int. J. Cancer* 134, 873 **(2014)**.

210. N. Rubinstein, M. Alvarez, N. W. Zwirner, M. A. Toscano, J. M. Ilarregui, A. Bravo, J. Mordoh, L. Fainboim, O. L. Podhajcer, and G. A. Rabinovich, Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection;

A potential mechanism of tumor-immune privilege. *Cancer Cell* 5, 241 **(2004)**.

211. M. Van Woensel, T. Mathivet, N. Wauthoz, R. Rosiere, A. D. Garg, P. Agostinis, V. Mathieu, R. Kiss, F. Lefranc, L. Boon, J. Belmans, S. W. Van Gool, H. Gerhardt, K. Amighi, and S. De Vleeschouwer, Sensitization of glioblastoma tumor micro-environment to chemo- and immunotherapy by Galectin-1 intranasal knock-down strategy. *Sci. Rep.* 7, 1217 **(2017)**.

212. G. Trinchieri, Biology of natural-killer cells. *Adv. Immunol.* 47, 187 **(1989)**.

213. M. Messaoudene, A. Frazao, P. J. Gavlovsky, A. Toubert, N. Dulphy, and A. Caignard, Patient's natural killer cells in the era of targeted therapies: Role for tumor killers. *Front. Immunol.* 8, 1 **(2017)**.

214. D. Gotthardt, E. M. Putz, E. Grundschober, M. Prchal-Murphy, E. Straka, P. Kudweis, G. Heller, Z. Bago-Horvath, A. Witalisz-Siepracka, A. A. Cumaraswamy, P. T. Gunning, B. Strobl, M. Muller, R. Moriggl, C. Stockmann, and V. Sexl, STAT5 Is a key regulator in NK cells and acts as a molecular switch from tumor surveillance to tumor promotion. *Cancer Discov.* 6, 414 **(2016)**.

215. G. S. Getz and C. A. Reardon, Natural killer T cells in atherosclerosis. *Nat. Rev. Cardiol.* 14, 304 **(2017)**.

216. A. Hamilton and N. R. Sibson, Role of the systemic immune system in brain metastasis. *Mol. Cell Neurosci.* 53, 42 **(2013)**.

217. Q. Yang, S. R. Goding, M. E. Hokland, and P. H. Basse, Antitumor activity of NK cells. *Immunol. Res.* 36, 13 **(2006)**.

218. C. Y. Dong, R. F. Mi, G. S. Jin, Y. Q. Zhou, J. Zhang, and F. S. Liu, Identification of the proliferative effect of Smad2 and 3 in the TGF beta 2/Smad signaling pathway using RNA interference in a glioma cell line. *Mol. Med. Rep.* 12, 1824 **(2015)**.

219. P. Roth, M. Mittelbronn, W. Wick, R. Meyermann, M. Tatagiba, and M. Weller, Malignant glioma cells counteract antitumor immune responses through expression of lectin-like transcript-1. *Cancer Res.* 67, 3540 **(2007)**.

220. E. A. Vasievich and L. Huang, The suppressive tumor microenvironment: A challenge in cancer immunotherapy. *Mol. Pharmaceut.* 8, 635 **(2011)**.

221. G. J. Kitange, B. L. Carlson, M. A. Schroeder, P. T. Grogan, J. D. Lamont, P. A. Decker, W. T. Wu, C. D. James, and J. N. Sarkaria, Induction of MGMT expression is associated with temozolomide resistance in glioblastoma xenografts. *Neuro. Oncol.* 11, 281 **(2009)**.

222. T. Kato, A. Natsume, H. Toda, H. Iwamizu, T. Sugita, R. Hachisu, R. Watanabe, K. Yuki, K. Motomura, K. Bankiewicz, and T. Wakabayashi, Efficient delivery of liposome-mediated MGMT-siRNA reinforces the cytotoxity of temozolomide in GBM-initiating cells. *Gene Ther.* 17, 1363 **(2010)**.

223. L. Gao, F. S. Li, B. Dong, J. Q. Zhang, Y. L. Rao, Y. Cong, B. Z. Mao, and X. H. Chen, Inhibition of Stat3 and Erbb2 suppresses tumor growth, enhances radiosensitivity, and induces mitochondria-dependent apoptosis in glioma cells. *Int. J Radiat. Oncol. Biol. Phys.* 77, 1223 **(2010)**.

224. X. P. Yuan, J. Du, S. Hua, H. W. Zhang, C. Gu, J. Wang, L. Yang, J. F. Huang, J. H. Yu, and F. J. Liu, Suppression of autophagy augments the radiosensitizing effects of STAT3 inhibition on human glioma cells. *Exp. Cell. Res.* 330, 267 **(2015)**.