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Immunosuppression in the microenvironment of glioblastoma
Imunosuprese v mikroprostředí glioblastomu

Bachelor´s thesis

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Podpis

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Abstract

Glioblastomas (GBM) are the most malignant brain tumors, which are thought to originate from neoplastic transformation of glial cells. These tumors are characterized with highly infiltrative growth, neovascularization, and radio- and chemoresistance. In spite of current therapy including surgical resection of the tumor and chemo/radio therapy, patient's prognosis is still poor and median survival is about 15 months. Certain non-tumor cells present in the GBM microenvironment participate in tumor progression using mechanisms contributing to the local and systemic immunosuppression. Critical roles in the immune escape of GBM have the regulatory T-cells (Tregs), the tumor-associated macrophages (TAMs) and the myeloid-derived suppressor cells (MDSCs). Immunosuppressive mechanisms in GBM are conducted through direct cell-mediated contacts and soluble mediators secreted by tumor-associated cells into the local tumor microenvironment and circulating blood. Both these processes may inhibit immune response mounted against cancer cells. Certain cancer associated cells and secreted mediators are distributed by peripheral blood and potentiate systemic immunosuppression in the GBM host organism. Gaining knowledge about these mechanisms may reveal to possible targets for GBM immunotherapy. For instance, therapeutic targeting of immune checkpoint molecules such as CTLA-4 and PD-L1 by inhibitors are now in the phase of clinical testing.

Key words

glioblastoma multiforme; tumor microenvironment; immunosuppression; immunotherapy; hypoxia; immune checkpoint molecules, Tregs, TAMs, MDSCs,

Abstrakt

Glioblastomy (GBM) jsou vysoce maligní mozkové nádory, které pravděpodobně vznikají neoplastickou transformací gliálních buněk. Vyznačují se vysoce infiltrativním růstem, hustou neovaskularizací a radio- a chemorezistencí. Současná terapie je založena především na chirurgickém odstranění nádorové léze a následné chemo- či radioterapii. Prognóza pacientů zůstává nadále velmi špatná a medián přežití je přibližně 15 měsíců. Buňky nádorového mikroprostředí se podílejí na progresi tumoru řadou mechanizmů, jejichž součástí je lokální i systémová imunosuprese. Tento efekt vykazují zejména regulační T-buňky (Tregs), nádorově asociované makrofágy (TAMs) a myeloidní supresorové buňky (MDSCs). Imunosupresivní mechanismy u GBM jsou vykonávány prostřednictvím přímých intercelulárních kontaktů a sekrecí solubilních mediátorů nádorově asociovanými buňkami do lokálního nádorového mikroprostředí a do cirkulující krve. Oba tyto procesy mohou inhibovat imunitní odpověď namířenou proti nádorovým buňkám. Pochopení těchto imunosupresivních mechanizmů může odhalit potenciální terapeutické cíle a metody vhodné k imunoterapii GBM. V současné době jsou ve fázi klinického testování inhibiční protilátky některých "immune checkpoint" molekul, a to například CTLA-4 a PD-L1.

Klíčová slová

glioblastoma multiforme; nádorové mikroprostředí; imunosuprese; imunoterapie; hypoxie; immune checkpoint molekuly**,** Tregs, TAMs, MDSCs,

List of abbreviations

APC - antigen presenting cells

ATRX - ATP-dependent helicase

BBB - blood brain barrier

CCL2 - chemokine C-C motif ligand 2

CCR4 - chemokine receptor type 4

CIC - homolog of the Drosophila gene capicua

gCSCs - glioma cancer stem cells

CSF-1 - colony stimulating factor 1

CTLA-4 - cytotoxic T-lymphocyte-associated protein 4

DCs - dendritic cells

ECM - extracellular matrix

EGFR - epidermal growth factor receptor

FasL - Fas ligand

FoxP3 - forkhead box P3

FUBP1 - far upstream element binding protein 1

GBM - glioblastoma

GBM IDH-M - glioblastoma with mutation in isocitrate dehydrogenase

GBM IDH-WT - glioblastoma with isocitrate dehydrogenase wild-type

HIF-1-α - hypoxia-inducible factor 1-α

HO-1 - heme oxygenase 1

IDH - isocitrate dehydrogenase

IDO - indoleamine 2, 3-dioxygenase

LLT1 - Lectin like transcript 1

LOH - loss of heterozygosity

MDSCs - myeloid-derived suppressor cells

MIC-1 - macrophage inhibitory cytokine 1

NK - natural killer cells

PD-L1 - programmed death-ligand 1

PTEN - phosphatase and tensin homolog

STAT3 - Signal transducer and activator of transcription 3

TAMs - tumor associated macrophages

TGF-β -transforming growth factor beta

TP53 - tumor protein p53

Tregs - regulatory T-cells

VEGF - vascular endothelial growth factor

WHO - the World Health Organization

Table of Contents

1. Introduction

One of the roles of immune system is to protect organism against cancer by a way of detection and elimination nascent tumor cells and by suppression of tumor growth. In some circumstances, immune system has insufficient capability to control tumor cells multiplication. Immune surveillance fails and this allows tumor cells to develop malignant tumors and to accomplish neoplastic progression. The functionality of immune system in the tumors has been a hot spotlight in scientific research for a few decades and glioblastoma is not an exception.

Glioblastoma multiforme (GBM) is the most common, aggressive and malignant brain tumor. Besides of its proliferative, invasive, and highly neovascularized characteristics, GBM have immunosuppressive properties consequent from the dynamic interactions of immune cells with the tumor microenvironment. GBM interact with immune cells at several stages to inhibit anti-tumor immune response and to mount the immunosuppressive microenvironment.

The GBM-mediated regulation of the immune system has arisen from the association of tumor cells with immune modulators. It promotes a strong immunosuppression in GBM microenvironment and leads eventually to tumor progression. The current therapy of GBM is based on the surgical resection of GBM lesion and on chemo- and radiotherapy. Understanding the immunosuppressive mechanisms which are generated in GBM microenvironment may lead to development of targeted GBM immunotherapy.

The aim of the thesis is to describe known mechanisms of immunosuppression within the glioblastoma microenvironment and to introduce some of these mechanisms as possible targets of immunotherapy.

2. Glioblastoma multiforme

Glioblastoma multiforme (GBM) is the most common and malignant brain tumor of central nervous system. GBM are formed from glial cells and the World Health Organization (WHO) classified them as glioma tumors grade IV. GBM are characterized by great cellular heterogeneity, aggressive invasion and high proliferation. Prognosis is poor and the median survival of patients is about 15 months (reviewed in Louis et al., 2016). WHO divided GBM in dependence to mutation in genetic marker isocitrate dehydrogenase (IDH) as IDH wild type (GBM IDH-WT) or mutant (GBM IDH-M) glioblastoma. GBM IDH-WT is predominant type in patients over 55 years and corresponds to clinical term – primary or *de novo* GBM. GBM IDH-M is most common in younger patients with history of prior lower grade gliomas. It is related to clinical term secondary GBM. Mutations in IDH are very rare in primary GBM but they exist and in that case, share comparable genetic features with secondary GBM, including tumor protein p53 (TP53) mutations and lacking epidermal growth factor receptor (EGFR) amplifications (Nobusawa et al., 2009). GBM IDH-WT is mostly developed from astrocytes or precursor stem cells and is highly heterogeneous with wide range of expression profiles detected as neural, pro-neural, classical, or mesenchymal (Verhaak et al., 2010). Typical genetic features are upregulation of vascular endothelial growth factor (VEGF) associated with spreading areas of necrosis, EGFR amplifications, mutation in phosphatase and tensin homolog (PTEN) and deficit of chromosome 10 (Godard et al., 2003; Lai et al., 2011; Ohgaki and Kleihues, 2007). Loss of heterozygosity 10q (LOH 10q) is a mutual genetic feature for primary and secondary GBM (Ohgaki et al., 2004).

Most of the GBM IDH-M develops from a glioma WHO grade II or anaplastic astrocytoma grade III (reviewed in Ohgaki and Kleihues, 2013). Typical genetic indicators of GBM IDH-M are mutations of IDH, ATP-dependent helicase (ATRX) and TP53 (Liu et al., 2012) (Figure1).

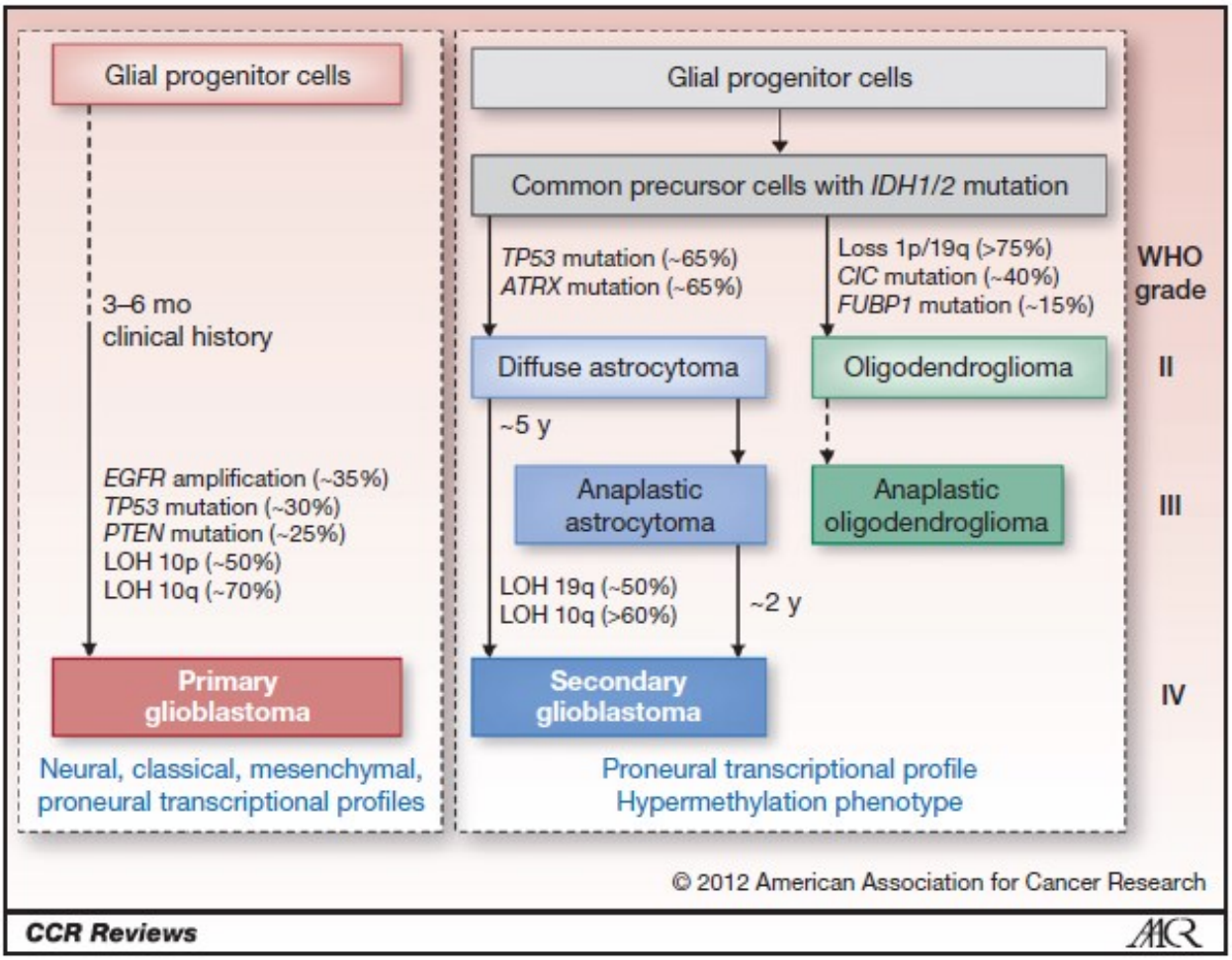

Figure 1: Genetic origins of primary and secondary GBM

EGFR (epidermal growth factor receptor), TP53 (tumor protein p53), PTEN (phosphatase and tensin homolog), ATRX (a-thalassemia/mental-retardation-syndrome-X-linked), CIC (homolog of the Drosophila gene capicua), FUBP1 (far upstream element binding protein 1), LOH (Loss of heterozygosity), IDH (isocitrate dehydrogenase) (reviewed in Ohgaki and Kleihues, 2013).

3. Glioblastoma microenvironment and immunosuppression

GBM contain tumor cells and non-malignant cells of the microenvironment participating in tumor development. Cells of immune system normally perform immune surveillance and monitor abnormal behaviors of cells and recognize tumor cells. Under GBM conditions, immune cells are converted into immunosuppressive phenotype or leading to apoptosis. Immunosuppressive behavior of cells in the microenvironment potentiate the proliferative and expanding characteristics of GBM including migration to borders of normal tissue and invasion, which is associated with degradation of extracellular matrix components (ECM). GBM are one of the most vascularized tumors. Overexpression of proangiogenic factors within tumor environment (e.g. VEGF) leads to impairment of endothelial cells, poor recruitment of pericytes and correlate with patient prognosis and survival (Plate et al., 1992). Circumstances associated with formation of new blood vessels promote hypoxic environment, necrotic areas, and disruption of blood brain barrier (BBB) integrity (On et al., 2013).

Blood flow disseminates immunosuppressive agents such as secreted factors and GBM related cells in body circulation. Suffering from GBM have not only strong local immunosuppressive defects but also systemic impact. Factors secreted in GBM microenvironment shift immune response to immunosuppressive Th2 supporting tumor progression (Kumar et al., 2006). In blood of patients bearing GBM some of the anomalies have been found in contrast to healthy people. Abnormal population of peripheral monocytes has been detected with large expansion in blood samples of GBM patients significantly defected about HLA-DR (molecule presenting antigens to T-cell) expression. In GBM patients, the expression of granulocyte macrophage colony-stimulating factor (GM-CSF), receptor necessary for monocytes development has been significantly decreased (Ogden et al., 2006). Monocytes with a lack of HLA-DR expression correlated with more unfavorable prognosis of GBM patients. Abnormal monocytes are unable to trigger signal in dendritic cells (DCs) to differentiate and activate T-helper cells (Gustafson et al., 2010; Woiciechowsky et al., 1998). Fraction of Tregs population has been increased in blood of GBM patients. Elevation of peripheral Tregs correlated with reduction of $CD4^+$ T-cells and their disability to function properly. Simultaneously with elevated Tregs, TGF-β, important factor for Tregs development, has been increased in patients serum as well (Fecci et al., 2006). MDSCs have also expanded in patient blood as result of dysfunctional and altered hematopoiesis in bone marrow. The activity of arginase is related to the increase of MDSCs

population in serum. Blood derived MDSCs have immunosuppressive effect on function of T-cells (Gielen et al., 2016).

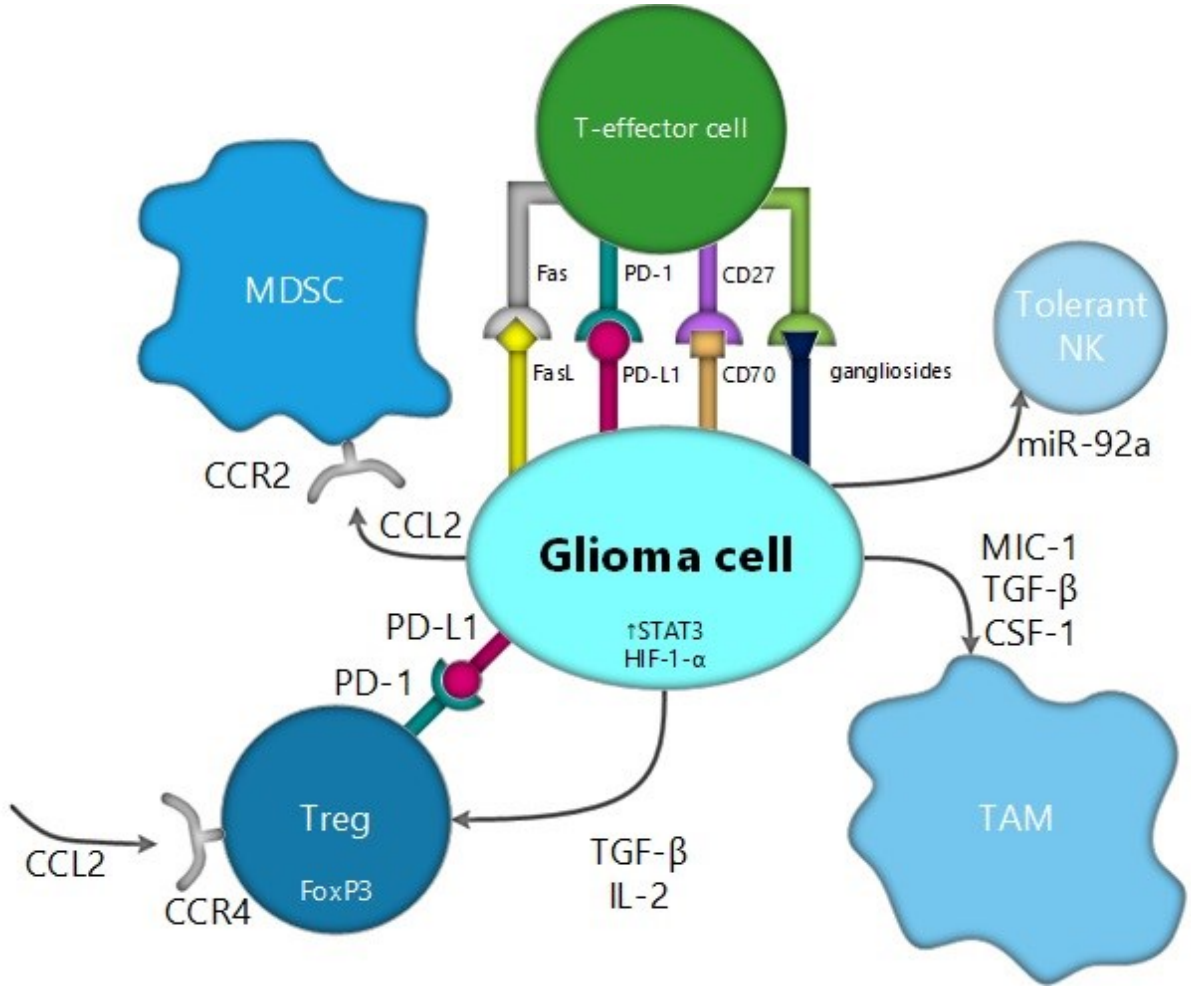

Figure 2: The immunosuppressive microenvironment of GBM

Infiltration and immunosuppressive phenotype of immune cells are maintained by many factors in GBM microenvironment. Polarization to M2 phenotype of tumor associated macrophages is mediated by GBM secreted factors such as macrophage inhibitory cytokine 1 (MIC-1), transforming growth factor (TGF-β) and colony stimulating factor 1 (CSF-1). Recruitment of Tregs is promoted by interaction of GBM's programmed death-ligand 1 and its receptor and secreted factors containing the chemokine ligand 2 (CCL2) binding with C-C chemokine receptor type 4 (CCR4) and TGF-β. Chemoattraction of MDSCs is mediated by the secretion of CCL2 in the microenvironment and interact with surface receptor of MDSCs C-C chemokine receptor type 2 (CCR2). Antitumor immune activity of natural killer cells (NK) is inhibited by tumor secreted miR-92a. Glioma cells have plentiful overexpressed surface antigens to inhibit activity of T-effector cells including Fas ligand (FasL), programmed death-ligand 1 (PD-L1), CD70 and gangliosides (Ben-Shoshan et al., 2008; Dong et al., 2002; Francisco et al., 2009; Chahlavi et al., 2005; Chang et al., 2016; Chen et al., 2003; Parney et al., 2000; Tang et al., 2014; Wu et al., 2010).

4. Mechanisms of immunosuppression in the microenvironment of GBM

GBM consist of cells which interact with immune system at many levels. They secrete factors triggering immunosuppressive phenotype of immune cells regulators or factors, which straightly affect activity of effector immune cells. Another way of GBM promoting immunosuppressive surroundings is through surface antigens by cell contact.

4.1. Hypoxia

Hypoxic environment is induced during tumor expansion, when new blood vessels with abnormal morphology are made as a consequence of necessary increasing oxygen utilization. Inhibitions of innate and adaptive immunity are both generated by hypoxia (Di Tomaso et al., 2010; Wei et al., 2010; Wu et al., 2010). Hypoxic environment is considered as an immunosuppressive condition and activates "Signal transducer and activator of transcription 3" (STAT3). Activation of STAT3 pathway in immune cells inhibits the secretion of stimulatory antitumor cytokines and contrariwise supports immunosuppressive activity with secretion of anti-inflammatory cytokines like interleukin 10 (IL-10) and transforming growth factor beta (TGF-β) (Kinjyo et al., 2006). Macrophages seem to be an important state with high-level of STAT3 activated pathway (Yaghi et al., 2017). Under hypoxic conditions, GBM are stimulated to produce VEGF with a role in neoangiogenesis and to simultaneously produce TGF-β and hypoxia-inducible factor 1-alpha (HIF-1-α) responsible for regulatory T-cells (Tregs) recruitment (Ben-Shoshan et al., 2008). Further cytokines that are triggered to be produced in association of hypoxia are colony stimulating factor 1 (CSF-1), chemokine C-C motif ligand 2 (CCL2) and galecitn-3 (Figure 2) (Brault and Kurt, 2005; Chang et al., 2016; Peng et al., 2008; Wu et al., 2010).

4.2. Immune checkpoint molecules

Immune checkpoint molecules take part in immunological evasion of GBM. The most important are programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and indoleamine-2, 3-dioxygenase (IDO).

The PD-L1 molecule (also known as B7-H1) is a critical immunoregulator of T-cell activation in GBM microenvironment. In the case of an immunosuppressive event, PD-L1 is located on the surface of glioma cells and is simultaneously expressed in TAMs and MDSCs (Bloch et al., 2013; Dong et al., 2002; Dubinski et al., 2016). The membrane-bound PD-L1

molecules are able to induce anergy of T-cells by counteracting TCR signaling, to suppress survival and to completely reduce the effector function of T-cells (Bloch et al., 2013). PD-L1 molecules on GBM's surface interact with PD-1 receptor molecules located on T-effector membrane generates and this triggers apoptotic response in T-cells (Figure 3) (Avril et al., 2010).

The CTLA-4 molecules are constitutively expressed in recruited Tregs and are upregulated in activated conventional T-cells (Takahashi et al., 2000). CTLA-4 molecules bind CD80 and CD86 molecules on the surface of antigen-presenting cells (APC) with higher affinity than does CD28 receptor of conventional T-cells. Hence, these receptor molecules compete for CD80 and CD86 binding. CD80 and CD86 molecules function as co-stimulators and are essential for generation of a full activating signal which leads to T-cell proliferation and terminal differentiation. Downregulation of immune response is mediated through outcompeting of CD28 co-stimulation and via depletion of CD80 and CD86 by endocytosis (Linsley et al., 1994; Qureshi et al., 2011). It has been demonstrated that the interaction of CTLA-4 with CD80/86 on DCs transmits signal that upregulates the overexpression of IDO and triggers its enzymatic activity (Munn et al., 2004).

The immunosuppressive role of **IDO** molecules in GBM is not fully elucidated. IDO is a cytosolic enzyme which degrades tryptophan to various metabolites which inhibit the proliferation of lymphocytes. The level of upregulated IDO in DCs is associated with the induction of Treg phenotype in naive T-cells (Fallarino et al., 2006). In addition, the increased expression of IDO is associated with worse prognosis in GBM patients (Wainwright et al., 2012).

4.3. Apoptosis of immune cells

Apoptosis is an essential biological process for keeping a healthy tissue by removal of old and damaged cells, followed by substitution with new cells. One of the approach contributes to apoptotic pathway is by **Fas ligand** (FasL) and Fas receptor interaction. The presence of transmembrane protein FasL has been confirmed in GBM cells and microglia as well. FasL interaction with Fas receptor of immune cells or microglia is a part of the processes leading to apoptosis of T-effector cells and contributes to the immunosuppressive milieu of GBM (Figure 3 and 4) (Badie et al., 2001; Parney et al., 2000). Further process transmitting apoptotic signal in T-cells is through CD70-CD27 interaction. CD70 is a tumor necrosis factor overexpressed on glioma cell surface (Yoon et al., 1999). Additional surface

molecules transmitting apoptotic signal in T-cells are gangliosides presented in GBM lines (Figure 3) (Chahlavi et al., 2005). The ability of gangliosides to inhibit immune response has been verified by the treatment of glucosylceramide synthase inhibitor and the competence to decrease infiltrating lymphocytes by apoptosis was significantly reduced (Chahlavi et al., 2005; Kudo et al., 2003).

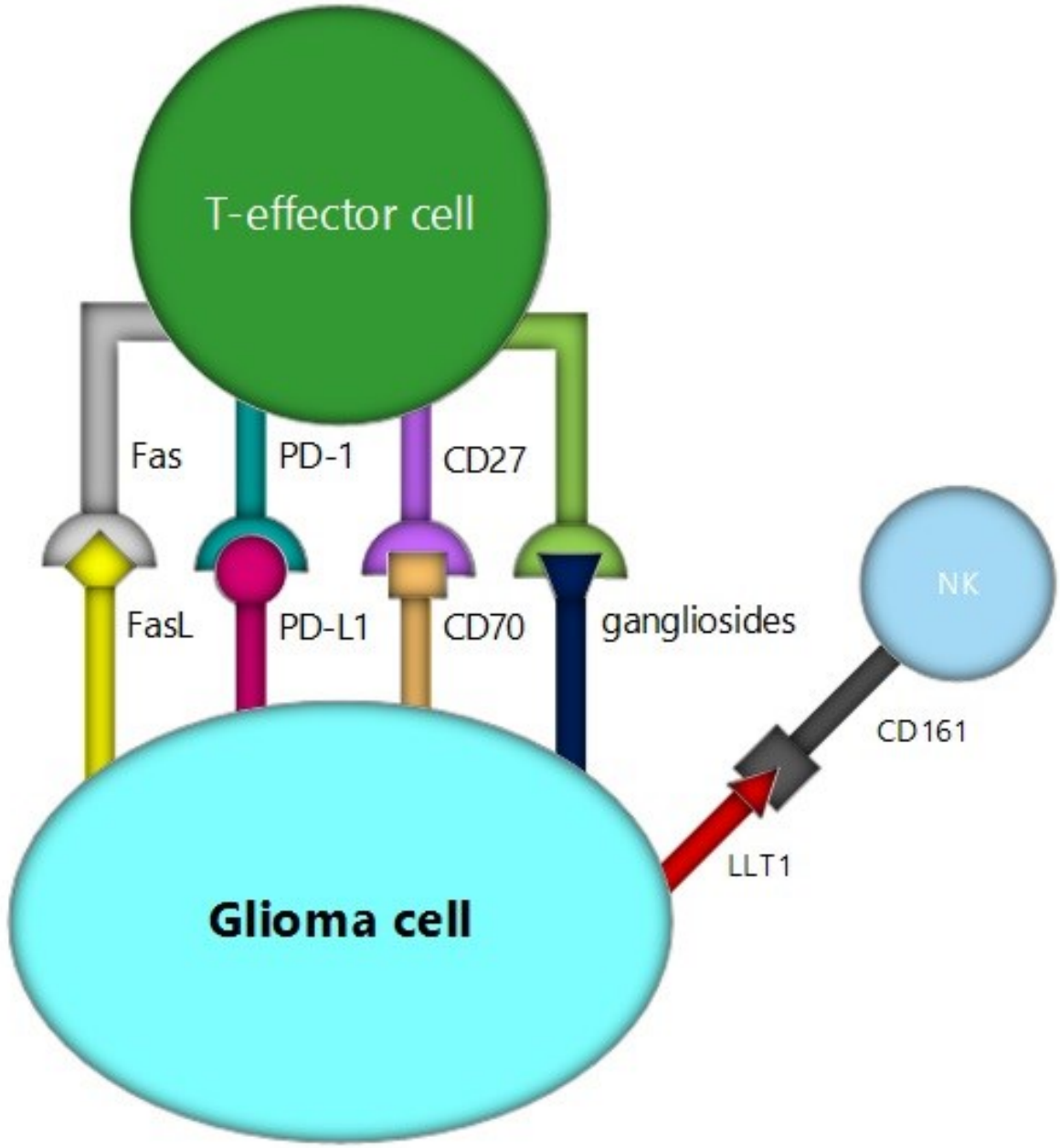

Figure 3: Immunosuppression promoted by glioma cell antigens

Glioma cells have plentiful overexpressed surface antigens to inhibit activity of T-effector cells including Fas ligand (FasL), programmed death-ligand 1 (PD-L1), CD70 and gangliosides. Furthermore, another GBM overexpressed antigen is Lectin like transcript 1 (LLT1), to bind natural killer cell receptor CD161 to suppress antitumor activity. (Dong et al., 2002; Chahlavi et al., 2005; Parney et al., 2000; Roth et al., 2007).

4.4. Phenotypic polarization of tumor associated macrophages and microglia

Tumor associated macrophages (TAMs) originating in macrophage lineage are immune cells presented in the surroundings of GBM. Macrophages and microglia with M1 phenotype stimulate inflammation by secretion of proinflammatory cytokines. On the contrary, M2 phenotype of macrophages and microglia decrease the inflammation. Shift to Th2 immune response stimulates secretion of anti-inflammatory cytokine IL-10 inhibiting production of pro-inflammatory cytokines like IFN-γ and TNF-α. Other important cytokines promoting tumor growth are IL-6 and IL-1 (Hao et al., 2002; Rodrigues et al., 2010). TAMs-secreted TGF-β1 increases the invasion capacity of tumors (Ye et al., 2012). After raised activity of STAT3 pathway in glioma cells, STAT3 activity in TAMs is increased as well and promotes M2 phenotype differentiation to support angiogenesis and tumor invasion and contributes to the immunosuppressive microenvironment of GBM (Lin et al., 2006). Glioma cancer stem cells (gCSCs) mediate the polarization of macrophages and microglia into immunosuppressive conditions by secretion of CSF-1, TGF-β1, and macrophage inhibitory cytokine 1 (MIC-1) (Figure 2). The phagocytic activity is inhibited but macrophages and microglia are stimulated to produce immunosuppressive cytokines IL-10 and TGF-β and their capacity to inhibit T-cell proliferation is enhanced (Wu et al., 2010) (Figure 3 and 4).

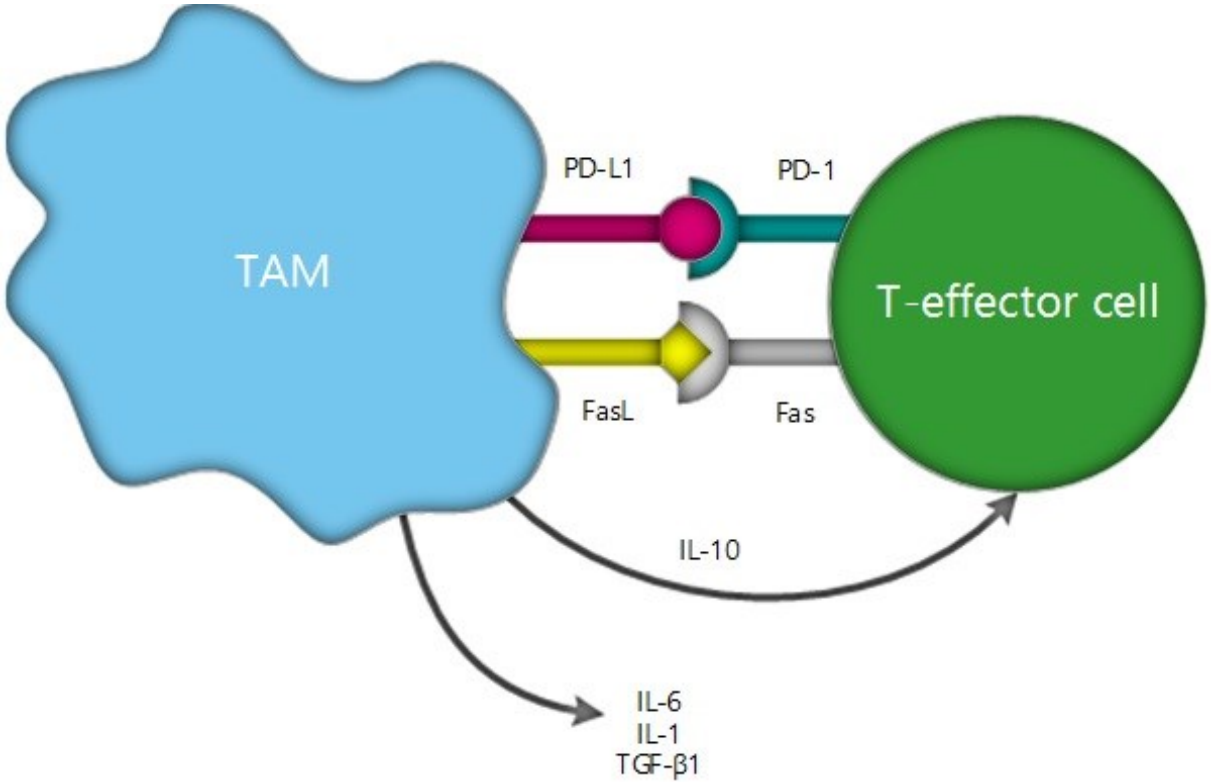

Figure 4: Immunosuppression promoted by Tumor associated macrophages (TAMs)

TAMs suppress activity of T-effector cells by overexpressed surface antigens programmed death-ligand 1 (PD-L1) and Fas ligand (FasL). Both antigens interact with their receptor PD-1 and Fas expressed by T-effector cells. Secreted cytokines IL-10, IL-6, IL-1 and TGF-β1 mediate immunosuppressive effect of the GBM milieu (Badie et al., 2001; Bloch et al., 2013; Hao et al., 2002).

4.5. Recruitment of regulatory T-cells

Regulatory T-cells (Tregs) have an immunosuppressive impact on the microenvironment of GBM and immune cells. Mechanisms of Tregs immunosuppression and recruitment are based on cell contact interactions or secretion of cytokines with immunosuppressive effects. Tregs mediated immunosuppression is more frequent in high-grade glioma originating in astroglioma than low-grade oligodendric gliomas (Heimberger et al., 2008).

The attraction of Tregs could be performed via hypoxic related STAT3 pathway inducing HIF-1-α production (Ben-Shoshan et al., 2008) and CCL2 signalization based on interaction with chemokine receptor type 4 (CCR4) of Tregs. The main sources of CCL2 are glial cells as well as macrophages (Chang et al., 2016). There are two distinct populations of Tregs in GBM microenvironment. Population of $CD4^+$ $CD25^+$ thymus-derived natural Tregs (nTregs) normally responsible for immune regulation derived *de novo* and induced Tregs (iTregs) differentiated from peripheral naive $CD4^+$ $CD25^-$ T-cells under GBM immunosuppressive conditions (Vasco et al., 2013). Conversion of peripheral naive T-cells to iTregs can be induced by secretion of TGF-β and IL-2. Concentration of TGF-β is significant for "forkhead box P3" (FoxP3) expression which is associated with Tregs differentiation (Chen et al., 2003). PD-L1 molecules overexpressed on the surface of glioma cells interact with PD-1 molecules of naive T-cells and interaction induces iTregs development and FoxP3 expression which has been demonstrated by *in vitro* experiment of Francisco et al. (2009) (Figure 2) (Francisco et al., 2009). Dominant population of $CD4^+CD25^+$Foxp3 cells presented in GBM are thymus-derived natural Tregs (Wainwright et al., 2011). Tregs are characterized by the expression of transmembrane immune checkpoint protein CTLA-4 to weaken immune response (Takahashi et al., 2000). GBM microenvironment induces IL-17^+Tregs to secrete cytokines IL-17 and TGF-β to promote inhibition of $CD8^+$ T-cells cytotoxic activity. Secreted TGF-β is a dominant molecule in Tregs to render immune suppressor capacity (Liang et al., 2014). Heme oxygenase-1 (HO-1) accumulates during glioma progression and the level of enzyme positively correlates with the frequency of FoxP3 mRNA expression in grade IV tumors. HO-1 is an enzyme with catalytic activity to degrade heme to biliverdin, ferrous iron and carbon monoxide (CO). Induction of HO-1 production in GBM is fomented by its hypoxic microenvironment. HO-1 molecules with enzymatic activity produce carbon monoxide to suppress T-cell proliferation. Expression of HO-1 enzyme is determined by the infiltration of Tregs population (Figure 4) (El Andaloussi and Lesniak, 2007).

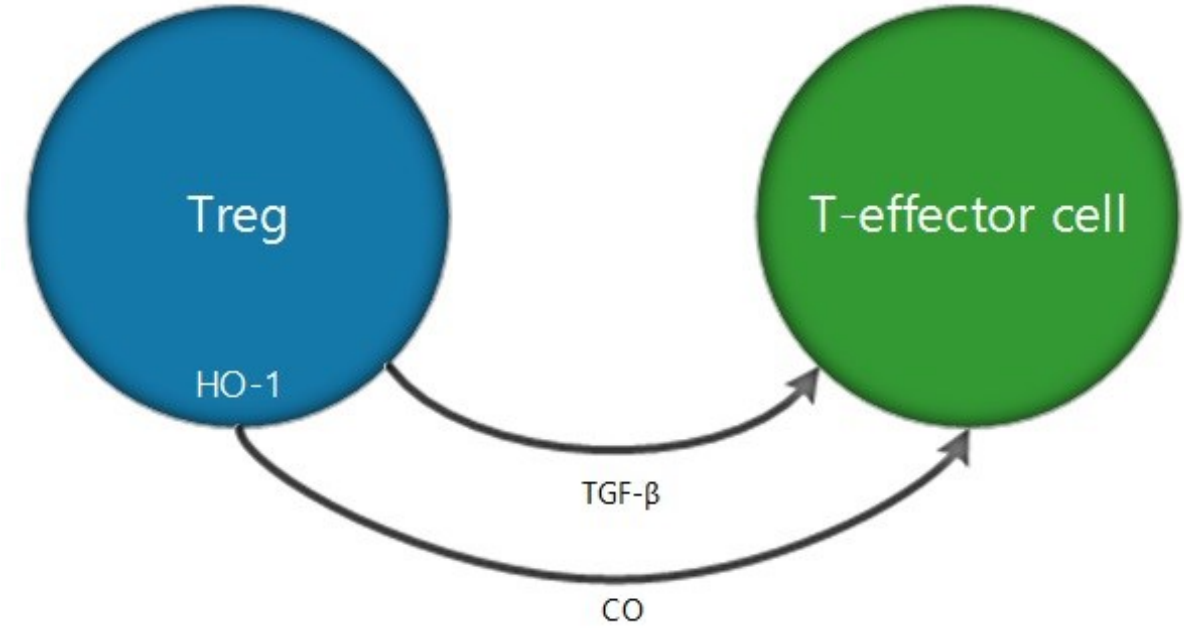

Figure 4: Immunosuppression promoted by regulatory T-cells (Tregs)

Tregs secrete immunosuppressive cytokine TGF-β and carbon monoxide produced by overexpressed enzyme heme oxygenase 1. Both processes induce anergy of T-effector cells and downregulation of immune response against cancer cells (El Andaloussi and Lesniak, 2007; Liang et al., 2014).

4.6. Differentiation of tolerant natural killer cells

Natural killer cells (NK) are cytotoxic type of lymphocytes which have origin in myeloid lineage. Mechanisms of their immune response are based on binding of activating and inhibiting receptors which recognize abnormal expression of MHC molecules, which is the case of tumor, and stimulate secretion of perforin and granzyme along with antitumor cytokines to attack tumor cells (reviewed in Vivier et al., 2011). GBM restrain antitumor mechanisms of NK and manage the tolerant NK differentiation. Immunosuppressive phenotype of tolerant NK is promoted via GBM secreted miR-92a. The ability of antitumor immune response is practically lost and NK are differentiated to inhibit $CD8^+$ T-cell activation and proliferation (Figure 2) (Tang et al., 2014). Overexpressed Lectin-like transcript 1 (LLT1) surface ligands in glioma cells interact with receptors CD161 of NK and inhibit specific antitumor activity of NK. By LLT1-CD161 interactions, the ability of NK recognizing stressed cells, including tumor cells, is downregulated and GBM immune evasiveness is supported (Figure 3) (Roth et al., 2007).

4.7. Infiltration of myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are heterogeneous type of cells originating in the myeloid lineage with immunosuppressive properties in GBM. There are $CD14^+$ monocytic and $CD15^+$ granulocytic MDSC populations presented in the microenvironment of GBM (Gielen et al., 2015). GBM induce secretion of various cytokines (e.g. VEGF, IL-6, GM-CSF and G-CSF) which positively influence the migration and activation of MDSCs to tumor microenvironment (Raychaudhuri et al., 2011). Moreover, MDSCs express the surface receptor CCR2 and thus they bind the chemokine CCL2, which is secreted by macrophages and glial cells. Chemoattractive effect of CCL2 stimulates MDSCs migration to GBM microenvironment (Figure 2) (Chang et al., 2016). MDSCs suppress the immune effector T-cells using several mechanisms which involve PD-L1 molecules (see above) and arginase I enzymes.

One of the mechanisms blocking T-cell activity is the upregulation of arginase I. This enzyme, when overexpressed in MDSCs that infiltrate GBM microenvironment, is responsible for depletion of the essential amino acid L-arginine, which is necessary for the expression of CD3ζ co-receptors and thus the function of T-cells. The deficiency of L-arginine in T-cells is the reason of a shorter mRNA half-life and lack of CD3ζ co-receptor biosynthesis (Figure 5) (Dubinski et al., 2016; Rodriguez et al., 2002).

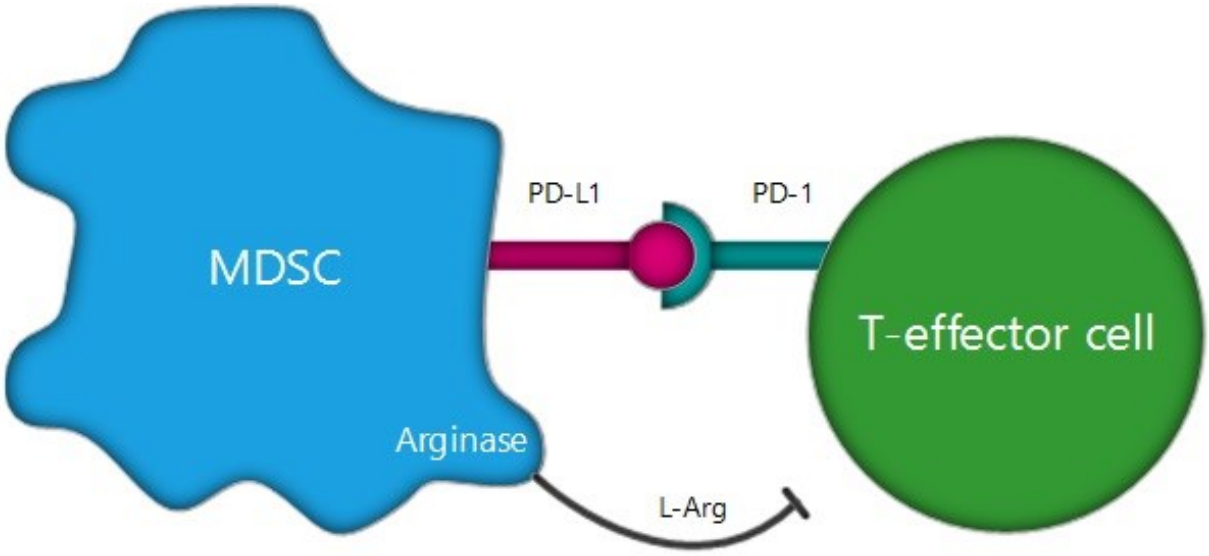

Figure 5: Immunosuppression promoted by myeloid-derived suppressor cell (MDSCs)

Overexpressed programmed death-ligand 1 (PD-L1) molecules in MDSCs interact with T-effector cell receptors PD-1 and mediate apoptopic response in T-cells. Upregulated arginase I enzyme degrades the amino acid L-arginine essential for T-cell development (Dubinski et al., 2016; Mazzoni et al., 2002).

5. Immune-based therapies

Successful therapy of GBM is limited by the immunosuppressive milieu and fail of immune response, which is not able to eliminate cancer cells. Immunotherapeutic approach is an appealing method for GBM treatment which focuses on stimulation of Th1 immune response and elimination of cancer cells.

Several studies were lead to develop drugs directed against various molecular markers such as EGFR, VEGF or PTEN either as monotherapy or in combination with other agents. However, the results from usage of inhibitors against genetic characteristics in GBM were generally weak and do not improve median survival or only slightly (Brown et al., 2016; Chinot et al., 2014; Ma et al., 2015).

Blockade of immune checkpoint molecules has brought exciting results of enhancing anticancer immune response in various types of tumor and provides a promising immunotherapeutical approach in newly diagnosed and recurrent GBM (Donin et al., 2017; Schadendorf et al., 2015; Slovin et al., 2013; Yang et al., 2007). There are several monoclonal antibodies targeting immune checkpoint molecules such as ipilimumab against CTLA-4, nivolumab and pembrolizumab targeting PD-1 and atezolizumab using against PD-L1. The idea coming from a perspective of using immune checkpoint molecules blockades, is that these drugs pass through BBB effectively and are active in CNS with an acceptable safety profile. Several clinical studies in I-III phases of trial are planned with inhibitors of immune checkpoint molecules up to 2020 (reviewed in Polivka et al., 2017).

Apart from clinical testing of inhibitors for immune checkpoint molecules, attention is focused on the other immunosuppressive mediators with possible potential in immunotherapy. There is study, which is aimed on depletion of Tregs. According to results of Curtin et al. (2008) Tregs elimination shows increase in survival of implanted mice. Even though Tregs depletion has some disadvantages, it looks like eradication of Tregs population is correlational with decrease of tumor antigen specific T-cells. Moreover, results from a depletion of Tregs show better expectations for survival only in a small-scale tumor (Curtin et al., 2008). Recent study of Yaghi et al. (2017) has come with an appealing approach of directly targeting STAT3 by developing of nanoparticle. Lipid nanoparticle containing miR-124 mostly contacts with circulating monocytes and macrophages which are known to be a location of STAT3 regulation. Nanoparticles containing miR-124 inhibit STAT3 translation after association with

monocytes and macrophages hence they function as professional antigen presenting cells and induce T-cell activation allowing anticancer response (Wei et al., 2013; Yaghi et al., 2017).

6. Conclusions

Glioblastomas (GBM) have strongly proliferative and highly invasive features which consequently lead to poor prognosis of patients suffering from these tumors, to high mortality and short median overall survival, despite of the contemporary treatment methods making it one of the most devastating types of cancers.

The pathogenesis and progression of GBM is dependent on the interaction of cancer cells with various components of GBM microenvironment including immune cells, playing an important role in the process immunosuppression. GBM are able to modulate the elements of immune system to induce immunosuppressive properties locally and also systemically. The intratumoral milieu of GBM promotes and governs the infiltration, recruitment and polarization of immune cells to get the immunosuppressive phenotype. The immunosuppression is accomplished via direct cell-cell interactions or through secretions of immunosuppressive factors to block anticancer immune response and it has a pivotal role in the tumor progression.

Targeting these immunosuppressive mechanisms gives us an opportunity to improve the effectiveness of GBM therapy. Currently, there are several promising ongoing trials of GBM immunotherapy based on the inhibition of immune checkpoint molecules.

7. References

Avril, T., Saikali, S., Vauleon, E., Jary, A., Hamlat, A., De Tayrac, M., Mosser, J., and Quillien, V. (2010). Distinct effects of human glioblastoma immunoregulatory molecules programmed cell death ligand-1 (PDL-1) and indoleamine 2,3-dioxygenase (IDO) on tumour-specific T cell functions. J Neuroimmunol *225*, 22-33.

Badie, B., Schartner, J., Prabakaran, S., Paul, J., and Vorpahl, J. (2001). Expression of Fas ligand by microglia: possible role in glioma immune evasion. J Neuroimmunol *120*, 19-24.
Ben-Shoshan, J., Maysel-Auslender, S., Mor, A., Keren, G., and George, J. (2008). Hypoxia controls CD4+CD25+ regulatory T-cell homeostasis via hypoxia-inducible factor-1alpha. Eur J Immunol *38*, 2412-2418.

Bloch, O., Crane, C.A., Kaur, R., Safaee, M., Rutkowski, M.J., and Parsa, A.T. (2013). Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. Clin Cancer Res *19*, 3165-3175.

Brault, M.S., and Kurt, R.A. (2005). Impact of Tumor-Derived CCL2 on Macrophage Effector Function. J Biomed Biotechnol *2005*, 37-43.

Brown, N., McBain, C., Nash, S., Hopkins, K., Sanghera, P., Saran, F., Phillips, M., Dungey, F., Clifton-Hadley, L., Wanek, K.*, et al.* (2016). Multi-Center Randomized Phase II Study Comparing Cediranib plus Gefitinib with Cediranib plus Placebo in Subjects with Recurrent/Progressive Glioblastoma. PLoS One *11*, e0156369.

Curtin, J.F., Candolfi, M., Fakhouri, T.M., Liu, C., Alden, A., Edwards, M., Lowenstein, P.R., and Castro, M.G. (2008). Treg depletion inhibits efficacy of cancer immunotherapy: implications for clinical trials. PLoS One *3*, e1983.

Di Tomaso, T., Mazzoleni, S., Wang, E., Sovena, G., Clavenna, D., Franzin, A., Mortini, P., Ferrone, S., Doglioni, C., Marincola, F.M.*, et al.* (2010). Immunobiological characterization of cancer stem cells isolated from glioblastoma patients. Clin Cancer Res *16*, 800-813.

Dong, H., Strome, S.E., Salomao, D.R., Tamura, H., Hirano, F., Flies, D.B., Roche, P.C., Lu, J., Zhu, G., Tamada, K.*, et al.* (2002). Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med *8*, 793-800.

Donin, N.M., Lenis, A.T., Holden, S., Drakaki, A., Pantuck, A., Belldegrun, A., and Chamie, K. (2017). Immunotherapy for the Treatment of Urothelial Carcinoma. J Urol *197*, 14-22.

Dubinski, D., Wolfer, J., Hasselblatt, M., Schneider-Hohendorf, T., Bogdahn, U., Stummer, W., Wiendl, H., and Grauer, O.M. (2016). CD4+ T effector memory cell dysfunction is associated with the accumulation of granulocytic myeloid-derived suppressor cells in glioblastoma patients. Neuro Oncol *18*, 807-818.

El Andaloussi, A., and Lesniak, M.S. (2007). CD4+ CD25+ FoxP3+ T-cell infiltration and heme oxygenase-1 expression correlate with tumor grade in human gliomas. J Neurooncol *83*, 145-152.

Fallarino, F., Grohmann, U., You, S., McGrath, B.C., Cavener, D.R., Vacca, C., Orabona, C., Bianchi, R., Belladonna, M.L., Volpi, C.*, et al.* (2006). The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J Immunol *176*, 6752-6761.

Fecci, P.E., Mitchell, D.A., Whitesides, J.F., Xie, W., Friedman, A.H., Archer, G.E., Herndon, J.E., 2nd, Bigner, D.D., Dranoff, G., and Sampson, J.H. (2006). Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res *66*, 3294-3302.

Francisco, L.M., Salinas, V.H., Brown, K.E., Vanguri, V.K., Freeman, G.J., Kuchroo, V.K., and Sharpe, A.H. (2009). PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med *206*, 3015-3029.

Gielen, P.R., Schulte, B.M., Kers-Rebel, E.D., Verrijp, K., Bossman, S.A., Ter Laan, M., Wesseling, P., and Adema, G.J. (2016). Elevated levels of polymorphonuclear myeloid-derived suppressor cells in patients with glioblastoma highly express S100A8/9 and arginase and suppress T cell function. Neuro Oncol *18*, 1253-1264.

Gielen, P.R., Schulte, B.M., Kers-Rebel, E.D., Verrijp, K., Petersen-Baltussen, H.M., ter Laan, M., Wesseling, P., and Adema, G.J. (2015). Increase in both CD14-positive and CD15-positive myeloid-derived suppressor cell subpopulations in the blood of patients with glioma but predominance of CD15-positive myeloid-derived suppressor cells in glioma tissue. J Neuropathol Exp Neurol *74*, 390-400.

Godard, S., Getz, G., Delorenzi, M., Farmer, P., Kobayashi, H., Desbaillets, I., Nozaki, M., Diserens, A.C., Hamou, M.F., Dietrich, P.Y.*, et al.* (2003). Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. Cancer Res *63*, 6613-6625.

Gustafson, M.P., Lin, Y., New, K.C., Bulur, P.A., O'Neill, B.P., Gastineau, D.A., and Dietz, A.B. (2010). Systemic immune suppression in glioblastoma: the interplay between CD14+HLA-DRlo/neg monocytes, tumor factors, and dexamethasone. Neuro Oncol *12*, 631-644.

Hao, C., Parney, I.F., Roa, W.H., Turner, J., Petruk, K.C., and Ramsay, D.A. (2002). Cytokine and cytokine receptor mRNA expression in human glioblastomas: evidence of Th1, Th2 and Th3 cytokine dysregulation. Acta Neuropathol *103*, 171-178.

Heimberger, A.B., Abou-Ghazal, M., Reina-Ortiz, C., Yang, D.S., Sun, W., Qiao, W., Hiraoka, N., and Fuller, G.N. (2008). Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. Clin Cancer Res *14*, 5166-5172.

Chahlavi, A., Rayman, P., Richmond, A.L., Biswas, K., Zhang, R., Vogelbaum, M., Tannenbaum, C., Barnett, G., and Finke, J.H. (2005). Glioblastomas induce T-lymphocyte death by two distinct pathways involving gangliosides and CD70. Cancer Res *65*, 5428-5438.

Chang, A.L., Miska, J., Wainwright, D.A., Dey, M., Rivetta, C.V., Yu, D., Kanojia, D., Pituch, K.C., Qiao, J., Pytel, P.*, et al.* (2016). CCL2 Produced by the Glioma

Microenvironment Is Essential for the Recruitment of Regulatory T Cells and Myeloid-Derived Suppressor Cells. Cancer Res *76*, 5671-5682.

Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003). Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med *198*, 1875-1886.

Chinot, O.L., Wick, W., Mason, W., Henriksson, R., Saran, F., Nishikawa, R., Carpentier, A.F., Hoang-Xuan, K., Kavan, P., Cernea, D.*, et al.* (2014). Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med *370*, 709-722.

Kinjyo, I., Inoue, H., Hamano, S., Fukuyama, S., Yoshimura, T., Koga, K., Takaki, H., Himeno, K., Takaesu, G., Kobayashi, T.*, et al.* (2006). Loss of SOCS3 in T helper cells resulted in reduced immune responses and hyperproduction of interleukin 10 and transforming growth factor-beta 1. J Exp Med *203*, 1021-1031.

Kudo, D., Rayman, P., Horton, C., Cathcart, M.K., Bukowski, R.M., Thornton, M., Tannenbaum, C., and Finke, J.H. (2003). Gangliosides expressed by the renal cell carcinoma cell line SK-RC-45 are involved in tumor-induced apoptosis of T cells. Cancer Res *63*, 1676-1683.

Kumar, R., Kamdar, D., Madden, L., Hills, C., Crooks, D., O'Brien, D., and Greenman, J. (2006). Th1/Th2 cytokine imbalance in meningioma, anaplastic astrocytoma and glioblastoma multiforme patients. Oncol Rep *15*, 1513-1516.

Lai, A., Kharbanda, S., Pope, W.B., Tran, A., Solis, O.E., Peale, F., Forrest, W.F., Pujara, K., Carrillo, J.A., Pandita, A.*, et al.* (2011). Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. J Clin Oncol *29*, 4482-4490.

Liang, H., Yi, L., Wang, X., Zhou, C., and Xu, L. (2014). Interleukin-17 facilitates the immune suppressor capacity of high-grade glioma-derived CD4 (+) CD25 (+) Foxp3 (+) T cells via releasing transforming growth factor beta. Scand J Immunol *80*, 144-150.

Lin, E.Y., Li, J.F., Gnatovskiy, L., Deng, Y., Zhu, L., Grzesik, D.A., Qian, H., Xue, X.N., and Pollard, J.W. (2006). Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Res *66*, 11238-11246.

Linsley, P.S., Greene, J.L., Brady, W., Bajorath, J., Ledbetter, J.A., and Peach, R. (1994). Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity *1*, 793-801.

Liu, X.Y., Gerges, N., Korshunov, A., Sabha, N., Khuong-Quang, D.A., Fontebasso, A.M., Fleming, A., Hadjadj, D., Schwartzentruber, J., Majewski, J.*, et al.* (2012). Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. Acta Neuropathol *124*, 615-625.

*Louis, D.N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W.K., Ohgaki, H., Wiestler, O.D., Kleihues, P., and Ellison, D.W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol *131*, 803-820.*

Ma, D.J., Galanis, E., Anderson, S.K., Schiff, D., Kaufmann, T.J., Peller, P.J., Giannini, C., Brown, P.D., Uhm, J.H., McGraw, S.*, et al.* (2015). A phase II trial of everolimus, temozolomide, and radiotherapy in patients with newly diagnosed glioblastoma: NCCTG N057K. Neuro Oncol *17*, 1261-1269.

Mazzoni, A., Bronte, V., Visintin, A., Spitzer, J.H., Apolloni, E., Serafini, P., Zanovello, P., and Segal, D.M. (2002). Myeloid Suppressor Lines Inhibit T Cell Responses by an NO-Dependent Mechanism. The Journal of Immunology *168*, 689-695.

Munn, D.H., Sharma, M.D., and Mellor, A.L. (2004). Ligation of B7-1/B7-2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. J Immunol *172*, 4100-4110.

Nobusawa, S., Watanabe, T., Kleihues, P., and Ohgaki, H. (2009). IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res *15*, 6002-6007.

Ogden, A.T., Horgan, D., Waziri, A., Anderson, D., Louca, J., McKhann, G.M., Sisti, M.B., Parsa, A.T., and Bruce, J.N. (2006). Defective receptor expression and dendritic cell differentiation of monocytes in glioblastomas. Neurosurgery *59*, 902-909; discussion 909-910.

Ohgaki, H., Dessen, P., Jourde, B., Horstmann, S., Nishikawa, T., Di Patre, P.L., Burkhard, C., Schuler, D., Probst-Hensch, N.M., Maiorka, P.C.*, et al.* (2004). Genetic pathways to glioblastoma: a population-based study. Cancer Res *64*, 6892-6899.

Ohgaki, H., and Kleihues, P. (2007). Genetic pathways to primary and secondary glioblastoma. Am J Pathol *170*, 1445-1453.

*Ohgaki, H., and Kleihues, P. (2013). The definition of primary and secondary glioblastoma. Clin Cancer Res *19*, 764-772.*

On, N.H., Mitchell, R., Savant, S.D., Bachmeier, C.J., Hatch, G.M., and Miller, D.W. (2013). Examination of blood-brain barrier (BBB) integrity in a mouse brain tumor model. J Neurooncol *111*, 133-143.

Parney, I.F., Farr-Jones, M.A., Chang, L.J., and Petruk, K.C. (2000). Human glioma immunobiology in vitro: implications for immunogene therapy. Neurosurgery *46*, 1169-1177; discussion 1177-1168.

Peng, W., Wang, H.Y., Miyahara, Y., Peng, G., and Wang, R.F. (2008). Tumor-associated galectin-3 modulates the function of tumor-reactive T cells. Cancer Res *68*, 7228-7236.
Plate, K.H., Breier, G., Weich, H.A., and Risau, W. (1992). Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature *359*, 845-848.

*Polivka, J., Jr., Polivka, J., Holubec, L., Kubikova, T., Priban, V., Hes, O., Pivovarcikova, K., and Treskova, I. (2017). Advances in Experimental Targeted Therapy and Immunotherapy for Patients with Glioblastoma Multiforme. Anticancer Res *37*, 21-33.*

Qureshi, O.S., Zheng, Y., Nakamura, K., Attridge, K., Manzotti, C., Schmidt, E.M., Baker, J., Jeffery, L.E., Kaur, S., Briggs, Z.*, et al.* (2011). Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science *332*, 600-603.

Raychaudhuri, B., Rayman, P., Ireland, J., Ko, J., Rini, B., Borden, E.C., Garcia, J., Vogelbaum, M.A., and Finke, J. (2011). Myeloid-derived suppressor cell accumulation and function in patients with newly diagnosed glioblastoma. Neuro Oncol *13*, 591-599.

Rodrigues, J.C., Gonzalez, G.C., Zhang, L., Ibrahim, G., Kelly, J.J., Gustafson, M.P., Lin, Y., Dietz, A.B., Forsyth, P.A., Yong, V.W.*, et al.* (2010). Normal human monocytes exposed to glioma cells acquire myeloid-derived suppressor cell-like properties. Neuro Oncol *12*, 351-365.

Rodriguez, P.C., Zea, A.H., Culotta, K.S., Zabaleta, J., Ochoa, J.B., and Ochoa, A.C. (2002). Regulation of T cell receptor CD3zeta chain expression by L-arginine. J Biol Chem *277*, 21123-21129.

Roth, P., Mittelbronn, M., Wick, W., Meyermann, R., Tatagiba, M., and Weller, M. (2007). Malignant glioma cells counteract antitumor immune responses through expression of lectin-like transcript-1. Cancer Res *67*, 3540-3544.

Schadendorf, D., Hodi, F.S., Robert, C., Weber, J.S., Margolin, K., Hamid, O., Patt, D., Chen, T.T., Berman, D.M., and Wolchok, J.D. (2015). Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. J Clin Oncol *33*, 1889-1894.

Slovin, S.F., Higano, C.S., Hamid, O., Tejwani, S., Harzstark, A., Alumkal, J.J., Scher, H.I., Chin, K., Gagnier, P., McHenry, M.B.*, et al.* (2013). Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol *24*, 1813-1821.

Takahashi, T., Tagami, T., Yamazaki, S., Uede, T., Shimizu, J., Sakaguchi, N., Mak, T.W., and Sakaguchi, S. (2000). Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med *192*, 303-310.

Tang, B., Wu, W., Wei, X., Li, Y., Ren, G., and Fan, W. (2014). Activation of glioma cells generates immune tolerant NKT cells. J Biol Chem *289*, 34595-34600.

Vasco, C., Canazza, A., Rizzo, A., Mossa, A., Corsini, E., Silvani, A., Fariselli, L., Salmaggi, A., and Ciusani, E. (2013). Circulating T regulatory cells migration and phenotype in glioblastoma patients: an in vitro study. J Neurooncol *115*, 353-363.

Verhaak, R.G., Hoadley, K.A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M.D., Miller, C.R., Ding, L., Golub, T., Mesirov, J.P.*, et al.* (2010). Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell *17*, 98-110.

*Vivier, E., Raulet, D.H., Moretta, A., Caligiuri, M.A., Zitvogel, L., Lanier, L.L., Yokoyama, W.M., and Ugolini, S. (2011). Innate or adaptive immunity? The example of natural killer cells. Science *331*, 44-49.*

Wainwright, D.A., Balyasnikova, I.V., Chang, A.L., Ahmed, A.U., Moon, K.S., Auffinger, B., Tobias, A.L., Han, Y., and Lesniak, M.S. (2012). IDO expression in brain tumors increases the recruitment of regulatory T cells and negatively impacts survival. Clin Cancer Res *18*, 6110-6121.

Wainwright, D.A., Sengupta, S., Han, Y., and Lesniak, M.S. (2011). Thymus-derived rather than tumor-induced regulatory T cells predominate in brain tumors. Neuro Oncol *13*, 1308-1323.

Wei, J., Barr, J., Kong, L.Y., Wang, Y., Wu, A., Sharma, A.K., Gumin, J., Henry, V., Colman, H., Priebe, W.*, et al.* (2010). Glioblastoma cancer-initiating cells inhibit T-cell proliferation and effector responses by the signal transducers and activators of transcription 3 pathway. Mol Cancer Ther *9*, 67-78.

Wei, J., Wang, F., Kong, L.Y., Xu, S., Doucette, T., Ferguson, S.D., Yang, Y., McEnery, K., Jethwa, K., Gjyshi, O.*, et al.* (2013). miR-124 inhibits STAT3 signaling to enhance T cell-mediated immune clearance of glioma. Cancer Res *73*, 3913-3926.

Woiciechowsky, C., Asadullah, K., Nestler, D., Schoning, B., Glockner, F., Docke, W.D., and Volk, H.D. (1998). Diminished monocytic HLA-DR expression and ex vivo cytokine secretion capacity in patients with glioblastoma: effect of tumor extirpation. J Neuroimmunol *84*, 164-171.

Wu, A., Wei, J., Kong, L.Y., Wang, Y., Priebe, W., Qiao, W., Sawaya, R., and Heimberger, A.B. (2010). Glioma cancer stem cells induce immunosuppressive macrophages/microglia. Neuro Oncol *12*, 1113-1125.

Yaghi, N.K., Wei, J., Hashimoto, Y., Kong, L.Y., Gabrusiewicz, K., Nduom, E.K., Ling, X., Huang, N., Zhou, S., Kerrigan, B.C.*, et al.* (2017). Immune modulatory nanoparticle therapeutics for intracerebral glioma. Neuro Oncol *19*, 372-382.

Yang, J.C., Hughes, M., Kammula, U., Royal, R., Sherry, R.M., Topalian, S.L., Suri, K.B., Levy, C., Allen, T., Mavroukakis, S.*, et al.* (2007). Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother *30*, 825-830.

Ye, X.Z., Xu, S.L., Xin, Y.H., Yu, S.C., Ping, Y.F., Chen, L., Xiao, H.L., Wang, B., Yi, L., Wang, Q.L.*, et al.* (2012). Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-beta1 signaling pathway. J Immunol *189*, 444-453.

Yoon, Y., Ao, Z., Cheng, Y., Schlossman, S.F., and Prasad, K.V. (1999). Murine Siva-1 and Siva-2, alternate splice forms of the mouse Siva gene, both bind to CD27 but differentially transduce apoptosis. Oncogene *18*, 7174-7179.