CHAPTER TWO

Personalized therapy and stem cell transplantation for pro-inflammatory modulation of cancer stem cells microenvironment in glioblastoma: Review

Igor Bryukhovetskiy[a,b,*], Oleg Pak[c], Yuri Khotimchenko[a,b], Andrey Bryukhovetskiy[d], Aruna Sharma[e], Hari Shanker Sharma[e]

[a]Department of Fundamental Medicine, School of Biomedicine, Far Eastern Federal University, Vladivostok, Russia
[b]Laboratory of Pharmacology, National Scientific Center of Marine Biology, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia
[c]Medical Center, Far Eastern Federal University, Vladivostok, Russia
[d]NeuroVita Clinic of Interventional and Restorative Neurology and Therapy, Moscow, Russia
[e]International Experimental Central Nervous System Injury & Repair (IECNSIR), Department of Surgical Sciences, Anesthesiology & Intensive Care Medicine, University Hospital, Uppsala University, S-75185 Uppsala, Sweden
[*]Corresponding author: e-mail address: igbryukhovetskiy@gmail.com

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International Review of Neurobiology, Volume 151
ISSN 0074-7742
https://doi.org/10.1016/bs.irn.2020.03.002

Abstract

Glioblastoma multiforme (GBM) is one of the most aggressive types of brain tumor in humans. The prognosis for patients with GBM is unfavorable and treatment is largely ineffective, where modern treatment regimens typically increase survival by 15 months. GBM relapse and progression are associated with cancer stem cells (CSCs). The present review provides a critical analysis of the primary reasons underlying the lack of effectiveness of modern CSC management methods. An emphasis is placed on the role of the blood-brain barrier in the development of treatment resistance. The existing methods for increasing the efficiency of antitumor genotoxic therapy are also described, and a strategy for personalized regulation of CSC based on post-genome technologies is suggested. The hypothesis that GBM cells employ a special mechanism for DNA repair based on their interactions with normal stem cells, is presented and the function of the tumor microenvironment in fulfilling the antitumor potential of normal stem cells is explained. Additionally, the mechanisms by which cancer stem cells regulate glioblastoma progression and recurrence are described based on novel biomedical technologies.

1. Introduction

Cancer is a fatal human disease, a longstanding scientific and clinical problem and a significant challenge for humanity. Despite advances in modern science, cancer remains one of the leading causes of mortality worldwide. Glioblastoma multiforme (GBM) is the most wide-spread primary malignant glial brain tumor.

Existing methods for treatment of GBM are ineffective. The standard complex treatment protocol yields a median survival of 15 months. Despite the efforts of medical professionals, only 25% of patents live for $\geq$2 years following diagnosis, whereas significant achievements have been made in the treatment of other types cancer in other locations (Stupp, Mason, van den Bent, et al., 2005; Stupp, Toms, & Kesari, 2016; Stupp & Weber, 2005).

GBM treatment resistance is associated with its cellular heterogeneity, as well as the presence of cancer stem cells (CSCs) with unique DNA repairing properties and the ability to initiate invasive growth and progression of the tumor shortly after surgical removal of the primary GBM nodule. There is no method for the effective eradication of CSCs in a patient's brain at present (Touat, Idbaih, Sanson, et al., 2017) and attempts at controlling CSCs with targeted drugs have proven unsuccessful. Together, this shows there are several systematic errors (Stupp, 2019) and critical gaps in the existing GBM

treatment protocols, which should be taken into consideration when developing novel approaches for treating patients with glial brain tumors.

2. Modern standards for GBM treatment and reasons for their inefficiency

In the 20th century, significant improvements were made in clinical oncology which were precipitated by the introduction of resection in surgical practice. Partial resection of an organ within healthy tissue or its radical removal altogether with adjacent tissue and regional lymph nodes have become a standard of clinical oncology and have increased the survival rates for patients with different types of cancer. The modern standard treatment for GBM is also surgical resection of the tumor. However, despite the active use of microsurgical devices, this is a non-radical surgery, as invasive GBM growth renders it impossible to remove the tumor completely without permanently damaging the patient.

GBM treatment primarily involves radiation and chemotherapy (Baumert, Hegi, van den Bent, et al., 2016). It is recommended that the patient receives 60 Gy of radiation (2 Gy daily, 30 fractions for 6 weeks) together with temozolomide (TMZ) chemotherapy (75 mg/m^2/day) (Nam & de Groot, 2017). High-dose radiation (Wegner, Abel, Horne, et al., 2019) is prescribed to the overwhelming majority of patients with GBM. An exception is only made for elderly patients, who can instead be treated with short-term radiation therapy of 40 Gy (15 fractions for 3 weeks) and chemotherapy (Braun & Ahluwalia, 2017; Perry, Laperriere, O'Callaghan, et al., 2017). But the patient's age is not necessarily a valid reason for dismissing aggressive treatment methods, and if a patient has a score >70 on the Karnofsky index, the standard method should still be adopted.

Increasing the radiation dose from 66 to 84.9 Gy does not significantly increase the patients' survival rates (Wegner et al., 2019). Intraoperative radiotherapy, CyberKnife, BNCT and injection of different types of nanoparticles into the brain to increase the efficiency of irradiation also do not increase the median survival of patients with GBM (Sulman, Ismaila, & Chang, 2017). Combining γ-radiation with proton beam therapy could escalate the radiation dose to 96.6 Gy and increase the survival time to 21 months (Mizumoto, Yamamoto, Ishikawa, et al., 2016; Mizumoto, Yamamoto, Takano, et al., 2015). However, increasing the radiation dose inevitably results in post-radiation necrosis that further damages the neural system.

The careful use of chemotherapeutic agents can extend the life of a patient with GBM. The most frequently selected drug for GBM treatment is TMZ which undergoes chemical conversion into an active metabolite in the body (Stupp et al., 2005, 2016; Stupp & Weber, 2005). This conversion is attributed to O6 and N7 guanine alkylation with a subsequent triggering of aberrant repair of the methyl adduct.

Usually patients are recommended to undergo 6–12 cycles of TMZ therapy. During the first cycle, 150 mg/m^2/day TMZ is administered on days 1–5 of a 28-day cycle, with the dose increased to $\leq$200 mg/m^2/day in subsequent cycles (Gilbert, Wang, Aldape, et al., 2013). Nevertheless, the increase in the number of chemotherapy cycles and the frequency of TMZ administration is not correlated with a significant improvement in survival rates (Blumenthal, Gorlia, Gilbert, et al., 2017). Documented attempts of extending the relapse-free period by combining TMZ with cisplatin (Wang, Kong, Guo, et al., 2017; Wang, Wang, Fu, et al., 2017), lomustine (Herrlinger, Tzaridis, Mack, et al., 2019; Stritzelberger, Distel, Buslei, et al., 2018), procarbazine, vincristine (Lassman, 2015), bevacizumab (Saran, Chinot, Henriksson, et al., 2016), cilengitide (Nabors, Fink, Mikkelsen, et al., 2015; Stupp, Hegi, Gorlia, et al., 2014), rindopepimut vaccine (Gerstner, 2017; Weller, Butowski, Tran, et al., 2017) and targeted drugs (Bryukhovetskiy, Bryukhovetskiy, Khotimchenko, et al., 2016; Bryukhovetskiy, Dyuizen, Shevchenko, et al., 2016; Bryukhovetskiy, Manzhulo, Mischenko, et al., 2016; Bryukhovetskiy, Shevchenko, Kovalev, et al., 2014; Stupp, 2019), have shown they were ineffective.

The efficiency of the existing GBM treatment methods are relatively low, and, despite clinicians' best efforts, patients inevitably relapse. Only 27% of patients live for 2 years following diagnosis. There are at least three reasons for this: the inability of drugs to get through the blood-brain barrier (BBB), the need for optimizing and personalizing the existing methods of GBM genotoxic therapy and the insufficient use of biomedical cellular technologies.

3. BBB and treatment resistance

It has been hypothesized that GBM damages the BBB and this hypothesis is primarily based on experiments showing the accumulation of gadolinium-based contrast in certain parts of the tumor in MRI scans (Vick, Khandekar, & Bigner, 1977). Intensive neoplastic angiogenesis disrupts the existing connections in the normal brain vasculature (Kane, 2019) and leads to edema of brain tissue. In turn, brain edema

damages the tight junctions between endothelial cells which results in the formation of pathological fenestrations, enabling the passive diffusion and intercellular transportation of drugs from the blood vessel to the neoplastic tissue (Wen, Tan, Dai, et al., 2017). However, new data has indicated that the BBB is only damaged in certain areas, particularly in the vessels surrounding certain parts of the tumor, and that the majority of GBM cells remains inaccessible to drugs (Dréan, Goldwirt, Verreault, et al., 2016; Oberoi, Parrish, Sio, et al., 2016; Sarkaria, Hu, Parney, et al., 2018).

Surgery results in extensive damage to the BBB, thus increasing the success of chemotherapy. However, solitary tumor cells that have migrated deeper into the brain tissue, subventricular zone (SVZ), corpus callosum, basal ganglia and other brain areas protected by the BBB, remain inaccessible. The low permeability of the BBB (Da Ros, De Gregorio, Iorio, et al., 2018) may thus explain the paradox between efficacy of various antitumor medications (Touat et al., 2017) which are effective against glioma and carcinoma cells *in vitro* but do not notably increase survival times in patients with GBM.

Experimental data has demonstrated the leading role of the BBB in treatment resistance. Osmotic or ultrasound damage of the BBB increases patient survival rates when combined with TMZ treatment compared with TMZ treatment alone (Oberoi et al., 2016). The suppression of multidrug resistance protein (Bulbake, Doppalapudi, Kommineni, Khan, et al., 2017; Tivnan, Zakaria, O'Leary, et al., 2015), breast cancer resistance protein (Martín, Sanchez-Sanchez, Herrera, et al., 2013), P-gp and other active-efflux transporters increases the efficiency of GBM treatment (Vries, Buckle, Zhao, et al., 2012). The permeability of the BBB to drugs is dependent on the environmental temperature and blood pressure (Sharma, Muresanu, Mössler, et al., 2019). Thus, the notion of creating prodrugs which are able to accumulate in the brain and are later synthesized into the active compound has been suggested (Da Ros et al., 2018).

The BBB can be penetrated through surgical intracerebral implantation, and intraventricular administration of drugs. However, these methods of drug administration are rarely implemented in clinical practice. Additionally, BBB permeability increases with the use of methamphetamine (Kiyatkin & Sharma, 2016), propofol (Sharma, Pontén, Gordh, et al., 2014) and a nano-drug delivery system (Sharma, 2011; Xu, Jia, Singh, et al., 2016).

4. Nano-based drug delivery

The use of modern nanosystems is one of the most effective, promising and safe methods for targeted delivery of anticancer drugs through the

BBB. Nanoparticles (NPs) can be defined as particles which are 1–1000 nm in size and possess colloidal properties. They potentially exhibit a range of possibilities for customization. NPs >200 nm in size are able to independently penetrate the brain through adsorption-mediated transcytosis, which allows them to be used for the delivery of therapeutics. NPs can be obtained from a wide range of natural and synthetic polymers and ~10 different types of materials are currently undergoing clinical trials for use of treatment for GBM. NPs being examined are based on polylactic glycolic acid (PLGA), polylactic acid (PLA), gold, silver and zinc oxide, as well as immunoliposomes.

PLGA is one of the most successfully used biodegradable polymers for drug delivery, as its hydrolysis in the body leads to the formation of lactic acid and glycolic acid, and these two monomers are readily metabolized by the body, and additionally PLGA has a low toxicity in the body (Danhier, Ansorena, Silva, et al., 2012; Danhier, Pourcelle, Marchand-Brynaert, et al., 2012). PLGA is approved by the US Food and Drug Administration and the European Medical Agency for various drug delivery systems. For example, doxorubicin-loaded PLGA NPs coated with Poloxamer 188 cross the BBB and effectively reduces tumor growth in a rat model (Wohlfart, Khalansky, Gelperina, et al., 2011). Paclitaxel encapsulated PLGA NPs significantly enhances the cytotoxic effect of the drug compared with taxol, which is 6 mg/mL paclitaxel-dissolved in a mixture of Cremophor® EL and ethanol (1:1) (Danhier, Lecouturier, Vroman, et al., 2009; Danhier, Vroman, Lecouturier, et al., 2009). PLGA NPs bound with an OX26 type monoclonal antibody against a transferrin receptor which is overexpressed in glioblastoma cells enhances the antitumor activity of TMZ on U215 and U87 GBM cell lines (Ramalho, Sevin, Gosselet, et al., 2018).

PLA is used less frequently than PLGA due to a reduced rate of decomposition. Numerous anticancer drugs were encapsulated in PLA-based NPs and evaluated *in vitro* and *in vivo* for the treatment of brain tumors. PLA NPs loaded with paclitaxel and conjugated with tLyp-1 peptide, a ligand with affinity to an overexpressed protein on glioma cells, significantly enhanced cellular uptake, increased the accumulation and penetration of paclitaxel in the target cells, and inhibited the progression of the tumor (Hu, Gu, Liu, et al., 2013; Liu, Huang, Ci, et al., 2017).

Polyethylene glycol is a polyether composed of repeated ethylene glycol units [-(CH_2CH_2O)$_n$]. Poly(ethylene glycol)-poly(ε-caprolactone) NPs functionalized with the LS10 peptide, which selectively binds to NG2

proteins (Chi, Zhu, Wang, et al., 2016), that are widely overexpressed in glioma cells, increased the absorption of paclitaxel in U87 cells and its penetration into spheroids. Functionalized NPs showed enhanced localization in the tumor, which resulted in an increase in the survival time of mice with glioma.

Chitosan is a copolymer of *N*-acetyl-glucosamine and *N*-glucosamine units linked by β-(1′4)-glycosidic bonds, and possesses good biocompatibility, biodegradability and low toxicity (Younes, Frachet, Rinaudo, et al., 2016; Younes & Rinaudo, 2015). Chitosan has been frequently modified to meet various biological and medical needs due to its active functional groups. A common modification method is chemical modification and the amino groups may be involved in chemical reactions, such as alkylation, quaternization, reacting with aldehydes and ketones. The hydroxyl functionality also leads to certain reactions such as *o*-acetylation, H-bonding with polar atoms and cross-linking (Sahariah & Másson, 2017). NPs based on stearic acid and chitosan (Thotakura, Dadarwal, Kumar, et al., 2017) have shown promising results for intravenous delivery of doxorubicin across the BBB (Xie, Du, Yuan, et al., 2012).

Alginates, derived from marine brown algae cell walls, are anionic, biodegradable and nontoxic polysaccharides consisting of a chain of 1-4-linked β-D-mannuronic acid and α-L-guluronic acid in different arrangements (Ching, Bansal, & Bhandari, 2017). Due to their anionic charge, alginates can be easily combined with chitosan, using electrostatic complexation. Alginate-chitosan NPs intranasal administration delivers the loaded doxorubicin primarily to the brain tissue with targeting efficiency reaching 480% (Hefnawy, Khalil, & El-Sherbiny, 2017).

Hyaluronic acid (HA) is a linear polysaccharide composed of D-glucuronic acid and *N*-acetyl-D-glucosamine. HA has been used for the development of targeted drug polymer conjugates for the treatment of brain tumors, particularly as it can be readily functionalized and conjugated with chemotherapeutics such as paclitaxel (Sofias, Dunne, Storm, et al., 2017) or doxorubicin (De Felice, Agostini, Suriano, et al., 2016). HA-based drug delivery systems provide targeted delivery of doxorubicin *via* CD44 receptors to GBM cells and increased antitumor activity (Hayward, Wilson, Kidambi, et al., 2016).

Lipidic NPs allow for the modulation of the release of drugs, their manufacturing does not necessarily require organic solvents and it can be easily scaled. Methods for the synthesis of various lipid NPs have been developed. Lipid nanocapsules are colloidal nanocarriers with a size of

10–1000 nm, the structure consists of a liquid core surrounded by a solid lipid shell, and possess the ability to effectively encapsulate hydrophobic and hydrophilic drugs (Nasr, Abdel-Hamid, & Alyoussef, 2015). Nanoliposomes are simpler than lipid NPs and are small nanometric vesicles (30–100 nm), consisting primarily of a phospholipid bilayer, which is formed by drying lipid film on water. They consist of an aqueous cavity and a hydrophobic membrane, that allows the administration of lipophilic and hydrophilic drugs (Zamani, Momtazi-Borojeni, Nik, et al., 2018). In addition, the phospholipids of liposomes can be combined with polyethylene glycol or polyethyleneimine molecules to increase the stability of the structure. The surface of the NPs, if necessary, can be functionalized with peptides, antibodies or any other ligands for active targeting (He, Lu, & Lin, 2015; Lu & Stenzel, 2018).

Several nanoliposome systems for treatment of cancer have shown promising results in preclinical and clinical studies on drug delivery and have been approved by the Food and Drug Administration (Bulbake et al., 2017). Liposomes are widely used to deliver anticancer drugs to GBM cells, including U87 and U118 cell lines and tumor neurospheres of human GBM (Belhadj, Ying, Cao, et al., 2017; Belhadj, Zhan, Ying, et al., 2017). The therapeutic effect of various doxorubicin-loaded liposome formulations has been studied in an orthotropic glioma model. It was found that liposomes functionalized with the cyclic signal sequence RGD and p-hydroxybenzoic acid exhibit a pronounced antiproliferative effect *in vitro*. Their use *in vivo* doubled the survival time of animals (Belhadj, Ying, et al., 2017; Belhadj, Zhan, et al., 2017).

The mechanisms for nanoliposome penetration through the BBB are usually associated with the presence of peptides on their surface or antibodies specific for receptors on the surface of barrier cells (Furtado, Björnmalm, Ayton, et al., 2018), for example, transferrin, insulin, lipoprotein and folate. Epirubicin liposomes modified with transferrin or tamoxifen significantly improved the effectiveness of the cytostatic drug in a rodent brain glioma model (Tian, Ying, Du, et al., 2010). Carmustine-loaded solid lipid nanoliposomes stimulate receptor-mediated transcytosis and increases the permeability of carmustine through the BBB (Kuo & Cheng, 2016). Insulin-coated nanoliposomes accumulate in the brain in increased quantities compared with other types of NPs studied. If packaged in nanoliposomes, drugs can also be delivered to the brain *via* cell transport, or they can be coated with red blood cell membranes (Chai, Sun, He, et al., 2017) to mask and pass through the BBB. Various inorganic NPs, such

as carbon, copper or iron oxide, gold, silver, titanium or silicon dioxide, can be used in diagnosing and treating brain tumors.

Carbon-based NPs are NPs consisting of graphite and its derivatives. Despite their poor solubility and cytotoxicity, they may be used as a means for drug delivery by functionalizing carbon NPs with various chemical compounds. For example, PEG-functionalized carbon nanotubes used in conjunction with immuno-adjuvant oligodeoxynucleotides CpG enhances the production of pro-inflammatory cytokines by primary monocytes that inhibit tumor growth and prolonged remission (Zhao & Nan, 2011).

In the last decade, there has been growing interest in the use of metal NPs in radiotherapy, due to their ability to absorb X-rays, and inorganic NPs with a high atomic number can exhibit radio-enhancing effects (Pinel, Thomas, Boura, et al., 2019). Due to the effect of increased permeability and retention, accumulation of NPs with a high atomic number in the tumor tissue allows for differentiation between the tumor tissue and healthy parenchyma. Thus, these effects together lead to an increase in absorbed radiation in the tumor, resulting in increased production of reactive oxygen species, increased DNA damage and the death of irradiated cells. Following intravenous injection with 11 nm gold NPs in mice with gliomas, gold NPs accumulated in the tumors with a tumor to healthy tissue ratio of 19:1. The accumulated NPs amounted to 1.5% of the tumor mass, which increased the calculated local radiation dose threefold (Hainfeld, Smilowitz, O'Connor, et al., 2013).

Strategies for transporting drugs through the BBB may vary, but any chemical substance, notwithstanding its efficiency *in vitro*, should only be considered for GBM treatment after developing technologies that can ensure their delivery through the BBB, and remain efficacious.

5. Genotoxic pharmacotherapy and methods for optimization

The primary mechanism by which existing cancer treatments exert their effects is by damaging the DNA of tumor cells (Erasimus, Gobin, Niclou, et al., 2016). Radiation and chemotherapy treatment for GBM results in alkylation of nitrogenous bases, single-stranded damage and double-stranded DNA breaks (Ciccia & Elledge, 2010; Kaina & Christmann, 2019). Tumor cell genome damage activates various DNA repair mechanisms, including direct DNA repair, base excision repair, homologous recombination and non-homologous end joining in GBM

cells. Inadequate DNA repair results in cell death as a result of mitotic catastrophe (Rycaj & Tang, 2014), critically high levels of genome instability (Kellner, DeMott, Cheng, et al., 2017) or the bystander effect (Widel, 2017).

The pleomorphism of molecular and genetic damage in GBM cells creates a multidirectional response to genotoxic therapy, which is one of the crucial mechanisms by which resistance develops. Patients with 1p/19q co-deletion or mutations of isocitrate dehydrogenase (IDH) in GBM cells are the most susceptible to genotoxic therapy (Nam & de Groot, 2017). IDH mutations result in excess 2-hydroxyglutarate levels in the cell, leading to the hypermethylation of the O6-methylguanine-DNA-methyltransferase (MGMT) gene, which is in turn responsible for direct DNA repair in cancer tumor cells. The median survival time of patients with GBM with MGMT hypermethylation is 23 months, whereas survival times in patients with MGMT hypomethylation is only 13 months (Bady, Kurscheid, Delorenzi, et al., 2018). The BRCA1/2 gene mutation prevents the repair of double-stranded DNA breaks by homologous recombination and increases the levels of endogenous stress (Rasmussen, Gajjar, Tuckova, et al., 2018), thus improving the efficiency of radiation and chemotherapy. Therefore, the suppression of DNA repair mechanisms should be a priority to increase the survival rates of patients with GBM.

There is a limited range of pharmaceutical agents available for the prevention of DNA repair. Lomeguatrib inhibits direct DNA repair (Taspinar, Ilgaz, Ozdemir, et al., 2013) but exhibits poor efficiency. The active metabolite of PF403 suppresses MGMT expression in TMZ-resistant GBM (Ji, Wang, Chen, et al., 2018). Methoxyamine prevents base excision DNA repair (Khoei, Shoja, Mostaar, et al., 2016). Olaparib combined with TMZ and radiation therapy initiates double-stranded DNA breaks that increase genome instability and result in death of GBM cells (Fulton, Short, James, et al., 2017).

Olaparib showed promising results when combined with TMZ and radiation therapy (Lesueur, Lequesne, Grellard, et al., 2019). Additionally, the effect of olaparib can be enhanced through combination with other inducers of chromosome instability, such as paclitaxel, gemcitabine, dactyloidae, cisplatin, peloruside A (Kim, Lee, Lee, et al., 2016; Lee, Lee, Kouprina, et al., 2016), oncolytic viruses (Ning, Wakimoto, Peters, et al., 2017), vorinostat (Galanis, Anderson, Miller, et al., 2018), duocarmycin SA (Blumenthal et al., 2017), inhibitors of key components of the mitochondrial respiratory chain (Goellner, Grimme, Brown, et al., 2011) and derivatives of marine

alkaloid fascaplysin (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016).

Therefore, a combination of radiation and chemotherapy, combined with antagonists of DNA repair, may improve the survival rates of patients with GBM. However, without eliminating or reducing the threat of CSCs, these treatments will be limited in improving overall survival.

6. CSCs and personalized therapy

CSCs present a significant challenge for successful treatment of cancer. These cells were first identified in tumors of hematopoietic and lymphoid tissues, breast cancer and glial brain tumors. CSCs are believed to hold an important place in the tumor cell hierarchy (Bradshaw, Wickremesekera, Brasch, et al., 2016; Bradshaw, Wickremsekera, Tan, et al., 2016); they are immortalized, function independent of external signals, are multipotent (Reya, Morrison, Clarke, et al., 2001), and exhibit high levels of proliferation and possess very accurate DNA repair abilities (Danhier, Ansorena, et al., 2012; Danhier, Pourcelle, et al., 2012).

The role of CSCs in the pathogenesis of GBM and the unique set of characteristics they possess, make CSCs an important target of all existing methods, including methods under development, for malignant brain tumors. The properties of CSCs have been described as cells which are immunopositive to at least one of the following, CD133, CD44, CD15, Olig2, Sox2, Stat3, Oct4 and Nanog (Gabrusiewicz, Li, Wei, et al., 2018; Inocencio, Frenster, & Placantonakis, 2018). Theoretically speaking, all these cells should possess the entirety of the properties attributed to CSCs; however, experimental data has demonstrated the unfounded nature of such statements.

CD133 is a glycoprotein and the most well-known CSC marker. $CD133^+$ cells in GBM which were extracted from gliomaspheres are characterized by a high rate of proliferation *in vitro* (Brown, Filiz, Daniel, et al., 2017); implanting 100 $CD133^+$ cells into the brains of experimental animals was sufficient for tumor development (Singh, Clarke, Hide, et al., 2004; Singh, Hawkins, Clarke, et al., 2004). However, the high rate of proliferation of $CD133^+$ cells is not a permanent trait. A study showed that the proliferation of tumor cells slows down due to hypoxia, leading to extensive necrosis in the center of the glioma nodule, while at the periphery, single clones of neoplastic cells, not always positive for $CD133^+$, continue

proliferating (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016).

After being implanted into an animal brain, $CD133^+$ cells create low-grade invasive tumors, similar to metastasis in structure. Reversible transitions between $CD133^+$ and $CD133^-$ cancer cells are possible (Li, Zhou, Xu, & Xiao, 2013), and these cells have a limited response to radiation (Brown, Daniel, D'Abaco, et al., 2015). The presence of the CD133 antigen in different types of cancer does not necessarily guarantee robust resistance to genotoxic therapy or enhanced DNA repair properties (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016; Bryukhovetskiy, Ponomarenko, Lyakhova, et al., 2018).

The CD44 antigen is another marker considered to be an indicator of an invasive CSC phenotype. However, at least partly, this phenotype arises from epithelial-mesenchymal transition (Goffart, Lombard, Lallemand, et al., 2017; Pińa Batista, Vega, de Eulate-Beramendi, et al., 2015) which is the result of a switch from adhesive E-cadherins to migratory N-cadherins, expanding the repertoire of integrin receptors expressed on the cell surface, as well as increasing interactions between these cells with the extracellular matrix and the production of extracellular matrix components and the matrix metalloproteinases MMP2, MMP9, MMP14 and ADAMTS1 (Bryukhovetskiy & Shevchenko, 2016). The presence of the CD44 antigen in GBM cells is not a determining factor of these changes, but a derivative one. If $CD44^+$ cells are transplanted into an experimental animal, they rapidly initiate invasive processes in a brain, but due to their low proliferative activity, the rate at which they produce tumors is relatively slow and thus are vulnerable to genotoxic therapy.

The hypothesis that double-positive $CD133^+CD44^+$ GBM cells exhibit increased proliferation, are the most motile cells and exhibit total resistance to treatment is unfounded (Brown et al., 2015). A cell with a markedly upregulated signaling domain of focal adhesion, which is actively migrating and synthesizing multiple components of extracellular matrix, will not have a high rate of proliferation. Additionally, there is a discrepancy between the large amounts of energy that cells require for these changes and the rather limited energy supply of the hypoxia-associated metabolism of GBM cells. Furthermore, when cells are extracted from a patient's brain with GBM, their radiation and TMZ resistance may be the result of previous treatment regimens.

The functions of other markers are also complicated. Membrane antigen CD15 is frequently referred to as a CSC marker. However, based on their

proliferative activity, cytogenetic characteristics and gene expression profile, $CD15^+$ GBM cells are similar to $CD15^-$ GBM cells (Kenney-Herbert, Al-Mayhani, Piccirillo, et al., 2015). $CD15^+$ and $CD15^-$ GBM cells form heterogeneous tumors in similar capacities, and the possibility of reversible transitions between the cells makes their CSC status questionable. Of note, surface antigen CD15 and nuclear antigen Olig2 are most frequently expressed in $CD133^+$ GBM cells.

Embryonic stem cell factors Nanog, Stat3, Oct4 and Sox2 are expressed in GBM cells (Ougland, Jonson, Moen, et al., 2016), extracted from gliomaspheres. These proteins are involved in tumor cell proliferation, migration and transformation (Seo, Jeon, & Kim, 2017) and are considered an important feature of a certain progenitor-like GBM cell phenotype (Vaidya, Bacchus, Sugaya, et al., 2018). However, the presence of these antigens in GBM cells is not particularly associated with diagnosis, as they are frequently expressed in $CD133^+$ cells.

Therefore, none of the aforementioned immunocytochemical markers characterize CSC cells, which exhibit the entire set of functional properties attributed to CSCs. Only the $CD133^+$ antigen can be used to characterize GBM cells which currently possess or had previously possessed certain CSC properties. However, the intensive search for targets that can suppress $CD133^+$ GBM cells has also proven to be ineffective.

$CD133^+$ GBM cells have been shown to possess increased expression of β-catenin, which is a key component of the WNT-signaling pathway regulating the proliferation of all stem cells (Shevchenko, Arnotskaya, Korneyko, et al., 2019). The upregulation of integrins and focal adhesion signaling pathways in $CD133^+$ GBM cells makes their proteome similar to that of normal stem cells, but the suppression of these mechanisms stimulates neoplastic cell proliferation, while their upregulation activates invasive processes (Bryukhovetskiy et al., 2014).

Genotoxic therapy is accompanied by a notable increase in the heterogeneity of GBM cells, thus it may not be useful to concentrate on certain markers of cancer cells. DNA repair transforms the genetic and epigenetic landscapes of GBM cells. If the cell is able to sufficiently repair its DNA and has the ability to proliferate and create a stem cell line, then these cell should be considered a cancer stem cell.

Based on experience (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016; Bryukhovetskiy et al., 2014), the traditional combination of TMZ and targeted drugs is not sufficient to inhibit the growth of these cells.

TMZ may be effective only with the successful suppression of DNA repair mechanisms, but large-scale clinical trials showed that such suppression was mostly inefficient following targeted therapy (Touat et al., 2017). A possible solution to this is personalized treatment, based on complex genomic and transcriptomic analysis of biopsy samples, obtained from a patient's tumor.

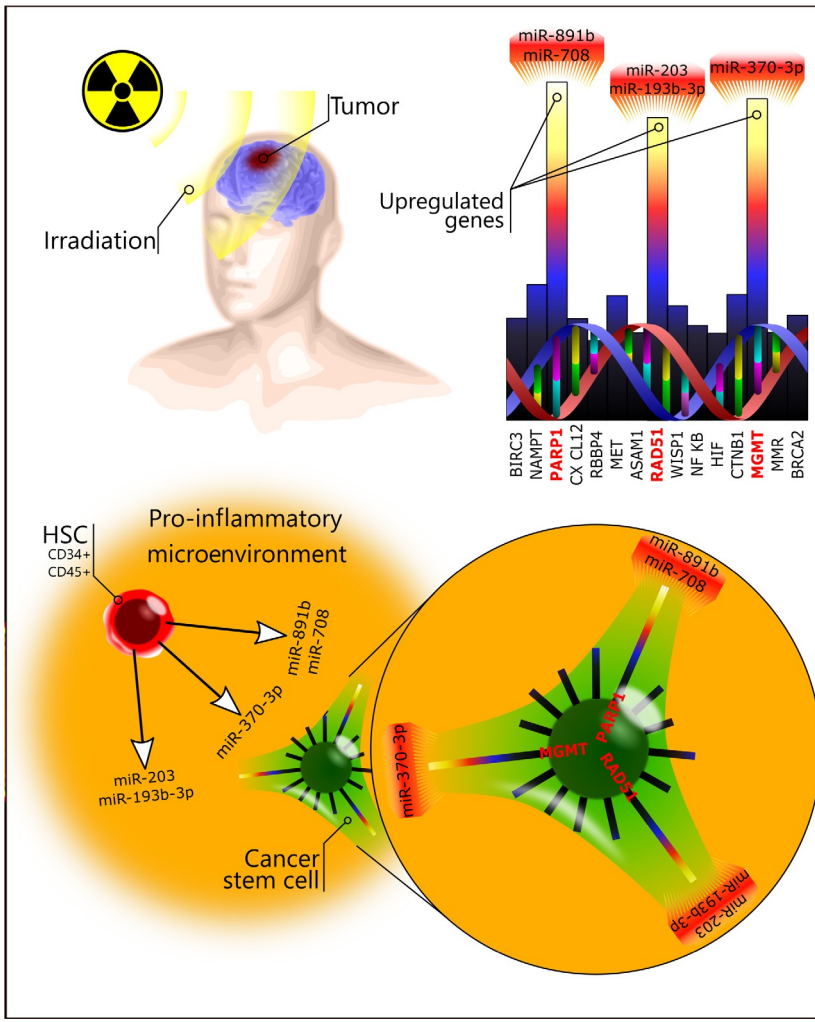

Fig. 1 Hypothetical scheme of target selection for the suppression of critically up-regulated genes in glioblastoma cancer stem cells. Red represents the critically upregulated genes that can be targeted using microRNAs.

There are a number of upregulated genes which have been associated with treatment resistance, including PARP1 (Murnyák, Kouhsari, Hershkovitch, et al., 2017), RBBP4 (Kitange, Mladek, Schroeder, et al., 2016), RAD 51 (Franceschi, Tomei, Mazzanti, et al., 2016; King, Brend, Payne, et al., 2017), NF-kB (Galardi, Mercatelli, Farace, et al., 2011), CXCL12 (Goffart et al., 2017), WISP1 (Jing, Zhang, Yu, et al., 2017), MET (De Bacco, D'Ambrosio, Casanova, et al., 2016) ASAH1 (Doan, Nguyen, Al-Gizawiy, et al., 2017), BIRC3 (Wang, Berglund, Kenchappa, et al., 2016), NAMPT (Gujar, Le, Mao, et al., 2016), HIF (Wang, Kong, et al., 2017; Wang, Wang, et al., 2017) and CTNNB1 (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016). The above list is not a comprehensive account of all the identified genes and there are likely many undiscovered genes which are upregulated and increase resistance to treatment; however, for the sake of brevity, only critically hyperexpressed genes which create Everest-like "peaks" above other upregulated genes are stated (Fig. 1). A promising method of inhibiting or reducing the expression of these targets is RNA interference with small RNA molecules (microRNAs) which could be used as a supplement for the existing cocktail of treatments used in various regimens for treatment of GBM. However, the suppression of critically upregulated genes is only one hurdle for complete resolution of GBM.

7. Normal stem cells and treatment resistance

High rates of mobility of normal stem cells toward GBM cells indicates their possible involvement in the repair of the tumor cell genome. Normal stem cells are a crucial component of the CSC niche and can have an inductive effect on each other. For the treatment of GBM, increased attention should be paid on neural and red bone marrow stem cells.

Since the first time neural stem and progenitor cells were extracted from an adult human brain (Altman, 1962), numerous parallels have been drawn between them and glioblastoma CSCs; however, successfully eliminating CSCs remains unresolved. Experimental data has shown there is an even more complicated connection between normal stem cells and CSCs than was previously thought. After implanting malignant glioma cells into an experimental animal brain (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016; Bryukhovetskiy et al., 2018), neural stem cells migrated from the SVZ, the subgranular zone of the dentate gyrus in the hippocampus and

other germinal brain centers to neoplastic lesions, where they adhered to tumor cells and interact with them.

GBM cells also migrate from the primary nodule to germinal brain centers, where they interact with neural stem cells, and this interaction tampers with the differentiation of neurons (Gao & Sun, 2019) and results in the preservation of a progenitor-like phenotype. Tumor growth within the SVZ has a considerably negative effect on the prognosis of patients with GBM (Altmann, Keller, & Schmidt, 2019). It is emblematic that after stereotactic introduction of normal neural stem cells into the tumor, these cells also follow cancer cells into the brain matter and adhere to and interact with them (Aboody, Brown, Rainov, et al., 2000).

The mechanisms underlying the interactions between GBM and neural stem cells differ. Excluding paracrine influences, the possibility of partial or complete fusion of interacting cells, or that cells form special bonds for the exchange of proteins, organelles and even cell nuclei, thus enabling epigenetic reprogramming of normal stem cells, has been described (Bastida-Ruiz, Van Hoesen, & Cohen, 2016). Evidence of this has been shown in studies showing complete and partial fusion of poorly differentiated cells when cultivating normal and CSCs *in vitro* (Bryukhovetskiy et al., 2018; Sontheimer, 2015).

However, if the interaction between GBM and neural stem cells produces special hybrids with a progenitor-like phenotype when migrating into the SVZ (Altman, 1962), theoretically these cells may use the rostral migratory stream to spread to the brain, which could be the primary reason underlying resistance to treatment (Singh, Clarke, et al., 2004; Singh, Hawkins, et al., 2004; Pińa Batista et al., 2015).

Bone marrow stem cell markers in a tumor indicate the severity of the disease (Milkina, Ponomarenko, Korneyko, et al., 2018). Glioblastoma produces >80 cytokines and chemoattractants, inducing bone marrow stem cell migration to the tumor lesion. The primary source of signaling molecules that attract normal stem cells to the tumor is hypoxia. Hypoxia results in the differentiation of a portion of CSCs of GBM into vascular epithelium and provides a supply of blood for the tumor (Hambardzumyan & Bergers, 2015), thus enabling the recruitment of hematopoietic and mesenchymal bone marrow stem cells. The recruited stem cells differentiate into vascular endothelial cells and produce vascular endothelial growth factor (VEGF) and thus become integrated into subsequent pathological processes.

Therefore, all normal stem cells exhibit potential to serve a special role in the pathogenesis of GBM, demonstrating their synergistic contribution to

the neoplastic process. The antitumor potential of normal stem cells has been shown in numerous studies. Studies have shown the ability of neural stem cells to suppress growth of poorly differentiated gliomas (Baklaushev, Grinenko, Savchenko, et al., 2012), the capability of normal hematopoietic $CD34^+CD45^+$ stem cells to regulate cancer cell proliferation and the potential of mesenchymal stem cells to express the top 10 microRNAs associated with the highest survival rates in patients with GBM: miR-302c-3p, miR-592, miR-484, miR-1260a, miR-493-3p, miR-145-5p, miR-30a-5p, miR-483-5p, miR-514a-3 and miR-124-3p (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016; Bryukhovetskiy et al., 2018; Mercatelli, Galardi, & Ciafrè, 2017).

The antitumor potential of normal stem cells is likely determined by influences from the local tumor microenvironment, which itself is the result of active participation of well-differentiated non-tumor cells.

8. CSCs and well-differentiated non-tumor cells in GBM

Well-differentiated non-tumor cells are the second group of cells which creates the local microenvironment of CSCs. They include astrocytes, fibroblasts and multiple immunocytes, particularly macrophages, monocytes, T-lymphocytes and NK-cells. The activity of non-tumor cells in neoplastic lesions can be divided into two groups, pro-inflammatory and immunosuppressive (Sontheimer, 2015).

The pro-inflammatory effect is primarily exerted by astrocytes and resident microgliocytes in the brain. The former surrounds the tumor, demarcating the tumor, and secretes nitrogen oxide (Kikugawa, Ida, Ihara, et al., 2017) and monocyte chemoactive protein 1 (Martin, Nguyen, Grunseich, et al., 2017), modulating the migration of resident microglia. The latter accumulates in the area of invasive growth and enters GBM tissues, where they produce tumor necrosis factor α, nitric oxide synthase, interleukins 1β, 2, 6, 8, 12 and 23, and interferon γ, creating a pro-inflammatory microenvironment (Poon, Sarkar, Yong, et al., 2017) and thus resulting in the transformation of the tumor-recruited normal stem cells and monocytes (Gabrusiewicz et al., 2018) into pro-inflammatory M1-type microgliocytes.

M1-activated macrophages and dendrite cells present tumor antigens to CD4 cells, which is associated with an increase in the production of pro-inflammatory cytokines. These advanced cellular mechanisms which should protect the brain and destroy the tumor, ultimately exarcerbate the

development and progression of GBM (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016; Bryukhovetskiy et al., 2018).

The expression of toll-like receptors in CSCs is relatively low (Alvarado, Thiagarajan, Mulkearns-Hubert, et al., 2017), and these receptors activate the NF-kB signaling pathway and thus synthesis of inflammatory mediators. The production of interleukin 1β by microglia (Howland, Sandman, et al., 2017) stimulates corticotropin-releasing hormone production from the hypothalamus (Kostopoulou, Mohammad, Bartek, et al., 2018), which in-turn increases the production of adrenocorticotropic hormone and glucocorticosteroids. If hormones are used to manage cerebral edema, the subsequent reduction in inflammation may result in the introduction of CSC markers into the tumor.

The availability of microglia in the brain is finite. Resident macrophages of the nervous system are derived from the embryonic yolk sac and support the brain population by proliferating; usually, bone marrow cells outside the BBB do not take part in this process. The development of a brain tumor quickly depletes the available microglia, and the increase in synthesis of TGF-β in the tumor lesion results in enhanced immunosuppression, which deteriorates due to the recruitment of mesenchymal stem cells by the tumor.

Red bone marrow monocytes, recruited into the tumor lesion, undergo a transformation into tumor-associated macrophages (Fig. 2), which are activated through an alternative M2-pathway under the influence of the immunosuppressive environment (Gabrusiewicz et al., 2018); this process (Bryukhovetskiy et al., 2018) is accompanied by an increased production of TGF-β and other anti-inflammatory cytokines, suppressing microglia, dendrites and effector T-cells. In response to TGF-β production by M2-macrophages, GBM cells increase the synthesis of this cytokine, based on autocrine induction. TGF-β initiates epithelial-mesenchymal transition in GBM cells and stimulates local immunosuppression, a primary requisite for tumor progression (Valdor, García-Bernal, Bueno, et al., 2017). Stem cells in the immunosuppressive microenvironment produce VEGF and fulfill their differentiation potential through creating blood vessels for an adequate blood supply. Thus, it is the anti-inflammatory local microenvironment that determines the fate of normal stem cells recruited to the tumor site, and determines their interactions with CSCs. So, it is the anti-inflammatory local microenvironment that determines the fate of normal stem cells recruited to the tumor site, and sets the main vector of their interaction with CSCs.

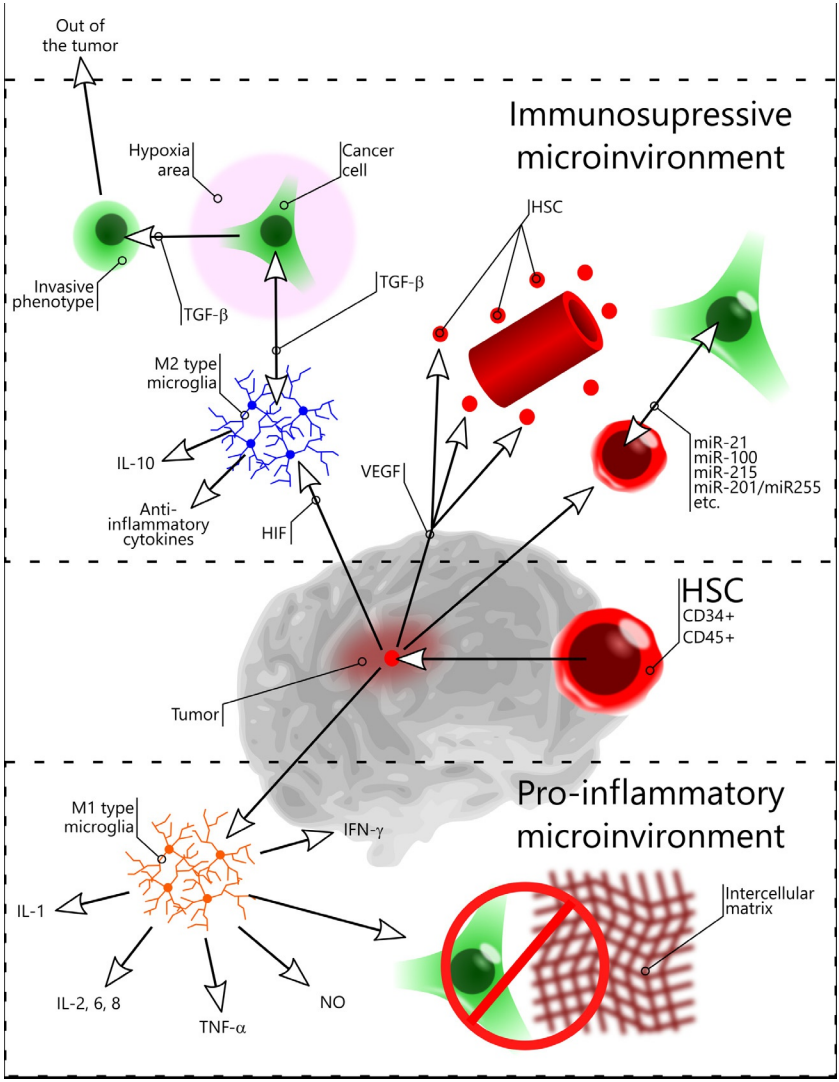

Fig. 2 Scheme determining the fate of a hematopoietic stem cell under the influence of the local microenvironment of the tumor. Left, the anti-inflammatory effect of the microenvironment: (i) VEGF production, angiogenesis and further recruitment of normal stem cells into the tumor; (ii) interactions with tumor cells, participation in genome repair, epigenetic reprogramming and the formation of cytohybrids; (iii) exchange between normal stem and tumor cells with oncogenic miRNAs. Right, pro-inflammatory effect of the microenvironment: differentiation into M1-microglia cells, production of pro-inflammatory cytokines, obstruction of invasive processes. VEGF, vascular endothelial cell growth factor.

9. Pro-inflammatory modification of the tumor microenvironment as a primary method of regulating glioblastoma cancer stem cells

Immunosuppression remains a major issue in oncology and is crucial for successful GBM treatment. Methods for systemic immunosuppression include, γ-radiation, cytostatics, cytotoxic agents and glucocorticosteroids. Long-term administration of high doses of TMZ and other chemotherapeutic agents is accompanied by the suppression of red bone marrow hematopoietic lineage and the development of infectious and hemorrhagic complications. The standard Stupp's protocol for the treatment of GBM (Stupp et al., 2005, 2016; Stupp & Weber, 2005) ignores this problem; however, experience has shown that regardless of the method or technology used, it will ultimately prove ineffective without resolving this issue first. The influence of the local immunosuppressive microenvironment of the tumor lesion predetermines the primary trajectory of the CSCs interactions with other cell types.

The immunosuppressive microenvironment, created due to the influence of TGF-β and other anti-inflammatory cytokines, contributes to M2-activated microglia and astroglia producing epidermal growth factor, Wnt5a and Wnt3a (Matias, Predes, Niemeyer Filho, et al., 2017) which activates the Wnt signaling cascade, a key molecular mechanism associated with proliferation and stemness. Wnt5a presence in the tumor tissue is associated with the degree of tumor infiltration, with microglia cells accounting for 30–50% of GBM cell mass (Dijksterhuis, Arthofer, Marinescu, et al., 2015; Du, Zhang, Chen, et al., 2017). Under the influence of Wnt proteins produced by anti-inflammatorily activated microglia cells, Wnt3a and Wnt5a production is increased by GBM (Binda, Visioli, Giani, et al., 2017; Kaur, Chettiar, Rathod, et al., 2013), thus, completing a "vicious circle," which ensures a rate of proliferation of CSCs and fast tumor relapse.

Another important factor for progression of GBM is the local remodeling of extracellular matrix (ECM) due to the presence of an immunosuppressive environment. These conditions promote active interaction of extracellular matrix components with CSCs and differentiated GBM cells with a mesenchymal phenotype, initiated by TGF-β (Bryukhovetskiy & Shevchenko, 2016), as well as the production of ECM components by these cells and their migration from the primary lesion to brain tissues.

Suppurative inflammation alone does not result in tissue malignancy in the majority of cases, since such inflammation is accompanied by total

dissolution of pathologically modified ECM. This suggests that total pro-inflammatory modification of the local microenvironment of GBM cells is a primary method of regulating and controlling CSCs. The positive effect of using chlorogenic acid, which induces the transition between M2 and M1 macrophages population along with suppression of arginase I and mannose receptor (CD206) production in the tumor together with simultaneous increase in production of iNOS and MHC class II molecules in these cells supports this hypothesis (Matias et al., 2017). Multiple studies from different laboratories showed promising results following injection of bacterial lipopolysaccharide and interferon γ in animals with glial brain tumors. These methods reveal the protective potential of macro- and microglia; however these methods may be insufficient given the overwhelming resources are relatively insufficient given the immunosuppressive abilities of the tumor. Immunosuppression regulates the proliferation and migration of cancer cells, promotes angiogenesis and recruitment of normal stem and differentiated cells into the neoplastic lesion and incorporates them into the oncogenic process, completing the "vicious circle" once again. A solution to this problem may only be found with the use of novel biomedical technologies.

Therefore, the pro-inflammatory modification of the local microenvironment is one of the dominant ways of regulating CSCs, as well as a major requirement for normal stem cells to fulfill their antitumor potential.

10. Novel biomedical technologies for treatment of GBM

A unique method of managing systemic immunosuppression is the transplantation of bone marrow stem cells. The most popular protocol in clinical practice involves the auto-transplantation of red bone marrow hematopoietic $CD45^+CD34^+$ stem cells, which have been recruited by granulocyte colony-stimulating factor into the blood flow. However, the antitumor potential of bone marrow cells is not limited to systemic immuno-correction.

When normal stem and progenitor bone marrow cells (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016; Bryukhovetskiy et al., 2018) are introduced into the blood flow of animals with poorly differentiated malignant glioma, they actively migrate to the tumor lesion, thus enabling their use for the targeted delivery of suicide genes and oncolytic viruses (Zhu,

Gorman, McKenzie, et al., 2017), radiosensitizers or pharmaceutical products. However, in the last 20 years there has not been any examples of the efficacy of such procedures for the treatment of GBM, highlighting the fact that these methods are primarily valuable for academic research purposes. The antitumor potential of normal stem cells could be most satisfactorily fulfilled for CSC management and the elimination of remaining tumor tissue (De Felice et al., 2016).

Allo- or auto-transplantation of bone marrow CD34+CD45+ cells combined with subsequent activation of a systemic inflammatory response by administrating bacterial lipopolysaccharide or interferon γ transforms the cells which migrated to the tumor into M1-pro-inflammatory microglia (Bryukhovetskiy et al., 2018). These cells actively produce pro-inflammatory cytokines, which thus presents an opportunity for managing the development of the tumor.

Auto-transplantation of the bone marrow can be performed directly after radiation and chemotherapy (Stupp et al., 2005, 2016; Stupp & Weber, 2005). However, it is not possible to treat every patient with autologous stem cells. A possible solution to this is the use of allogeneic bone marrow cells.

Relapse-free time and the quality of life for patients who received a high dose of chemotherapy and allotransplantation of bone marrow cells are significantly improved compared with patients who received autologous cells (Slavin, Naparstek, Nagler, et al., 1995; Wang, Kong, et al., 2017; Wang, Wang, et al., 2017). This may be based on the ability of the donor's allogeneic lymphocytes, an integral part of bone marrow $CD45^+$ cell fraction, to attack the remaining tumor tissue in the recipient's body (Weiden, Sullivan, Flournoy, et al., 1981).

Bone marrow allotransplantation is used both in myeloablative and non-myeloablative treatment regimens. In myeloablative regimens, apart from myelosuppression management, the therapeutic effect is dependent on the ability of the donor's T-lymphocytes to attack the remaining cancer cells in the patient's body (Gaffen & Liu, 2004). The therapeutic effect of bone marrow allotransplantation can be significantly enhanced with subsequent administration of allogeneic lymphocytes, activated by recombinant interleukin 2. Unfortunately, there are no clones of T-cells that are only aggressive toward tumor cells, and, even if hypothetically, they could be transduced through the use of suicide genes, the risk of undertaking such treatment could only be justified if the patients conditions is serious and would warrant such a potentially high risk procedure.

Excluding E-lymphocytes, the antitumor effects of allogeneic $CD45^+$ bone marrow cells is determined by the involvement of monocytes, macrophages and NK-cells ability to suppress neoplastic cells, regardless of the presence of antigens for the major histocompatibility complex. The transplantation of allogeneic bone marrow following TMZ chemotherapy significantly improved the survival rates of animals with poorly differentiated glioma (Bryukhovetskiy, Bryukhovetskiy, et al., 2016; Bryukhovetskiy, Dyuizen, et al., 2016; Bryukhovetskiy, Manzhulo, et al., 2016). However, increased inflammation in the post-surgical tumor bed is followed by cerebral edema which is considerably dangerous (Or-Geva & Reisner, 2015).

Non-myeloablative allotransplantation of bone marrow cells should be considered when hematopoietic stem cells of the recipient reject the donor's lymphocytes (Reisner, Gur, Reich-Zeliger, et al., 2005). In turn, hematopoietic stem cells of the donor reject all responsive cells of the host that have remained following chemotherapy. This creates a bilateral tolerance and a classic example of the regulatory function of hematopoietic stem cells.

Theoretically, subsequent controlled allotransplantation of bone marrow $CD34^+CD45^+$ cells, reinforced by immunocompetent T-cells, may provide stability in the local "transplant vs host" reaction, creating a pro-inflammatory environment around CSCs, which could prove to be a potential tool for the management of these cells.

11. Conclusion

GBM treatment is a significant challenge for clinicians, and novel antitumor therapies are required. The aim of the present review was to provide a critical analysis of the primary reasons for the lack of success of GBM treatment. The primary issue is BBB penetrability. The notion of CSC immunocytochemical polymorphism is debated, and the use of RNA interference combined with traditional genotoxic GBM treatments against considerably upregulated genes in the CSCs of a patient with GBM should be considered. The function of normal stem cells and differentiated cells in the pathogenesis of GBM is described, and the hypothesis of a predominant fulfillment of normal stem cells' anticancer potential in local pro-inflammatory microenvironment has been suggested. The necessity of modulation of the overall and local immunosuppression in complex GBM therapy is presented, and methods for achieving this with new biomedical technologies are described.

The present review therefore systematically describes major approaches and defined strategies for the creation of new methods for the treatment of invasive malignant glial brain tumors.

Funding

This study was funded by the Ministry of Science and Higher Education of Russia (Grant no. 14.584.21.0027 ID: RFMEFI58417X0027).

Authors' contributions

IB wrote the manuscript, proposed the study idea, designed the study, offered support with the experiments, organized the scientific team, performed an analysis of literature and own scientific reports, provided scientific guidance. YK and HS discussed, analyzed and interpreted the results of the study, and also worked on the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests

The authors declare that they have no competing interests.

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