Brain Tumor Stem Cells and Immunotherapy

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Abstract: Glioblastoma multiforme (GBM) is one of the most common aggressive primary brain tumors, and it responds poorly to the current treatment combination of surgery, radio-, and chemotherapy. The hypothesis that cancer stem cells may account for the pathogenesis underlying various tumors, including GBM, has been accepted widely in recent years. Brain tumor stem cells (BTSCs) have been shown to contribute to therapeutic resistance and the presence of BTSCs may explain the recurrence of GBM following conventional treatment, as just a few BTSCs are sufficient to give rise to a new tumor. Therefore, the therapeutic targeting of BTSCs is of utmost importance. Among emerging treatment modalities, immunotherapy is a strategy that has the potential to target BTSCs that are resistant to conventional therapies. This review describes recent advancements in the study of BTSCs and immunotherapy.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most lethal primary brain tumor with a median survival of 14.6 months, even with aggressive therapy including surgery, radio-, and chemotherapy, due to tumor invasion, frequent recurrence, and resistance to conventional therapies [1]. The cancer stem cell (CSC) hypothesis, first proposed in 1997, explains not only the cellular and genetic heterogeneity, but also the poor prognosis of GBM [2, 3]. A few brain tumor stem cells (BTSCs) capable of self-renewal, multilineage differentiation, and resistance to conventional therapy are sufficient to give rise to recurrent tumors following treatment [4]. Despite continuing efforts to study BTSCs, an effective therapy that targets BTSCs has remained elusive. Immunotherapies for glioma include cytokine modulation, adoptive immunotherapy, and active immunotherapy. Recently, active immunotherapy using a peptide vaccine has shown encouraging results for high-grade glioma, and clinical trials are ongoing [5-10]. Moreover, the effectiveness of immunotherapy in targeting BTSCs has been demonstrated [11-13]. The latest findings related to BTSCs and immunotherapy are reviewed in this article.

1. BRAIN TUMOR STEM CELLS

CSCs are thought to be a subpopulation of tumor cells with the capacity for self-renewal and multilineage differentiation, resulting in the heterogeneity of cancer cells [14]. CSCs were first identified in acute myeloid leukemia in 1997 [2]. The subsequent identification of CSCs in brain tumors, prostate cancer, colon cancer, breast cancer, pancreas cancer, ovary cancer and melanoma established the important role of CSC in the tumorigenesis of several cancer types.

1.1. Identification of Brain Tumor Stem Cells

The first identification of BTSCs in GBM was reported in 2003 [3]. BTSCs exhibit many similarities to normal neural stem cells (NSCs). The characteristics of BTSCs are the ability to generate clonally derived cells, self-renew, and the potential to differentiate into multiple lineages (neural, astrocytic, and oligodendroglial) [15]. They also express Nestin and Sox2 that are thought to be NSC markers [4]. Initially, BTSCs were cultured as spheroids in serum-free media containing the epidermal growth factor (EGF) and the fibroblastic growth factor (FGF) [4, 16]. Lee *et al.* reported that tumor cells maintained in NSC culture conditions could be stably preserved and form tumors similar to parental tumors in immunodeficient mice [4]. Presently, as culture methods have diversified, NSCs are cultured *in vitro* as spheres, or under adherent conditions in two-dimensional cultures or three-dimensional matrices. Sphere-forming assays that were initially used to culture NSC are not the only procedure to establish NSCs and are considered to have limitations in evaluating NSCs when removed from their *in vivo* environment [17]. In addition, in CSC cultures, the sphere-forming assay is not the only assay to isolate CSCs as it is suggested to reflect the distinct behavior of cells outside of their original environment.

1.2. Molecular Markers

The identification and purification of BTSCs from human GBMs and medulloblastomas were first

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reported by Singh *et al.* and CD133 was thought to be an important BTSC marker [16]. In this report, as few as 100 CD133+ cells were enough to form xenograft brain tumors in immunocompromised mice, and histologically, these tumors resembled the patient's tumor. In contrast, as many as 1×10^5 CD133- cells could not generate tumors [16]. However, other groups have reported that CD133- tumor cells isolated from GBMs have displayed stem-like cell properties [18, 19]. A more recent report suggested that CD133- cells may result in brain tumor initiation and that CD133- cells can generate a CD133+ cell population [20]. It is still unclear whether CD133 is a marker of BTSCs and whether it is associated with tumor initiation. Other stem cell markers have also been suggested [21-23]. Son *et al.* demonstrated that stage-specific embryonic antigen 1 (SSEA-1; CD15)-positive GBM cells fulfill the functional criteria for BTSCs [21]. In this report, SSEA-1+ cells were highly tumorigenic *in vivo* and exhibited self-renewal and multilineage differentiation. Integrin α6 has also been reported as a BTSC marker. Lathia *et al.* demonstrated that targeting integrin α6 in BTSCs inhibited self-renewal, proliferation, and tumor formation capacity [22]. Anido *et al.* reported that BTSCs in GBM express high levels of CD44 and the inhibitor of DNA-binding protein 1 (Id1) [23]. Inhibition of the transforming growth factor-β (TGF-β) pathway decreased the CD44/Id1 high population and prevented tumor initiation and recurrence. Still, the specific BTSC marker has not been discovered. It is important to discover a reliable BTSC marker because it would allow effective enrichment and targeting of BTSCs. Further studies about BTSC markers are ongoing (Table **1**).

1.3. Signal Transduction

Various proteins and signaling pathways present in BTSCs have been reported [24-33]. These include transcription factors such as oligodendrocyte lineage transcription factor 2 (Olig2), Nanog, and Bmi1, receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), signaling pathways involved in neural development such as Sonic Hedgehog (SHH), Notch, and major pathways regulating survival and proliferation. In addition, other pathways such as the TGF-β/BMP pathway, have been reported to be associated with differentiation of BTSCs, [34-36].

Olig2 is suggested to be involved in the proliferation of neural progenitors, in addition to glioma formation. Ligon *et al.* showed that Olig2 can regulate the lineage-restricted pathway critical for proliferation of normal and tumorigenic neural stem cells [24].

Nanog is a transcription factor that controls the stemness of embryonic stem cells, and is expressed in several human cancers [37]. The SHH-Gli1 pathway regulates the proliferation of neural progenitor cells [38] and may have an important role in the self-renewal and tumor growth of glioma BTSCs [25-29].

Bmi1 was originally identified as an oncogene involved in the induction of lymphoma [39, 40], and has been shown to be essential for the proliferation and self-renewal of NSCs [41]. In an orthotropic transplantation model of glioma, Bmi1 was reported to be required for tumor development and regulates BTSCs [30].

The Notch pathway is important in the biology of normal NSCs as well as BTSCs [31, 42]. Elevated Notch signaling enhances the efflux of cytotoxic drugs through ABC transporters such as ABCG2, thereby contributing to the resistance of BTSC to conventional therapies.

The PI3K-AKT-mTOR pathway is the major signaling pathway activated by receptor tyrosine kinases (RTK), including EGFR and PDGFR in GBM [43], and is a cell survival pathway. In GBM and medulloblastoma, this pathway results in stem-like behavior [32]. Furthermore, activation of this signaling

Table 1: Brain Tumor Stem Cell Markers

BTSC marker	BT type	Reference
CD133	GBM, MB	[16]
SSEA-1	GBM	[21]
Integrin α6	GBM	[22]
CD44/Id1	GBM	[23]

BTSC, brain tumor stem cell; BT, brain tumor; SSEA-1, stage-specific embryonic antigen 1; Id1, inhibitor of DNA-binding protein 1; GBM, Glioblastoma; MB, Medulloblastoma.

through PTEN loss leads to the proliferation of BTSCs [33].

TGF-β and their family members, including bone morphogenetic proteins (BMPs), have been demonstrated to play important roles in maintenance and differentiation of normal stem cells. TGF-β pathway was reported to be essential for BTSCs in glioma to retain their stemness and inhibition of TGF-β signaling may lead to differentiation of BTSCs [34]. BMP-4 has been shown to induce astrocytic differentiation and reduce tumorigenicity of BTSCs in glioma [35]. Lee *et al.* identified BMP type 1B receptor (BMPR1B) that induces GBM BTSCs to differentiate [36].

1.4. The BTSC Niche

Like NSCs, BTSCs are thought to require the specific microenvironment, termed "niche", for survival and self-renewal. BTSCs residing in the niche sustain tumor survival by being protected from external factors, and maintain their stemness by receiving some factors from within the niche. The important factors associated with the BTSC niche are thought to be angiogenesis and oxygen nutrition. It has been shown that BTSCs exist in a vascular niche and that coculture of BTSCs with endothelial cells enhanced tumor growth [44]. BTSCs themselves secrete vascular endothelial growth factor (VEGF) and promote angiogenesis [45]. In addition, 2 different reports suggest that BTSCs could transdifferentiate into endothelial cells directly [46, 47]. A hypoxic environment as part of the NSC niche is important for maintaining their self-renewal [48]. Hypoxia induced factor (HIF) is a transcription factor that functions as a master regulator of oxygen homeostasis, and depletion of HIFs in BTSCs is reported to inhibit their self-renewal, survival, and tumor initiation [49, 50]. HIFs also play an important role in upregulating VEGF signaling and promoting angiogenesis, resulting in maintenance of the tumor and its microenvironment.

1.5. Controversies of CSCs

Despite several efforts to characterize BTSCs, some inconsistent findings have been noted, as mentioned above. These contradictory results suggest that in contrast to normal NCSs, BTSCs may be heterogeneous. Moreover, the diversity of BTSCs might be a cause of GBM heterogeneity. BTSCs may not be a static population, but instead a dynamically modulated population due to its genomic instability, differentiation, and plasticity. Piccirillo *et al.* reported

the existence of distinct BTSCs in the same GBM [51]. The plasticity of CSCs was reported previously with respect to melanoma [52]; therefore, a similar theory may be true in the case of BTSCs.

Although CSCs were defined as cells that can initiate tumors *in vivo*, the current definition does not require the ability to form tumors upon implantation [53]. It was shown that tumor cells with a low expression of Id1, a stem cell marker, and those that were unable to form spheres, had a higher tumorigenicity in high-grade glioma [54]. Thus, the definition of CSCs is now being re-evaluated. Visvader pointed out the difference between tumor-initiating cells and CSCs [55]. The former corresponds to cells of origin that acquire the first cancer-promoting mutations, whereas the latter relates to tumor-propagating cells.

In summary, many unanswered questions regarding the characterization of BTSCs, including their markers, niche, and other defining factors, still remain. Further studies are necessary to improve our understanding.

2. GLIOMA AND IMMUNOTHERAPY

Currently, many clinical trials of immunotherapy for malignant gliomas are ongoing. We describe the basic concept of immunotherapy and then review recent studies on immunotherapy for GBMs and BTSCs.

2.1. Basic Immunology

The immune system has an important role in preventing foreign and dangerous antigens from damaging the body. Innate immunity involves an antigen-nonspecific response, while adaptive immunity is based on the antigen-specific response [56, 57]. B cells mediate antibody immunity and killer T cells play a main role in the cellular immune response. Helper T cells are involved in both forms of adaptive immunity. B cells recognize specific extracellular antigens and secrete antigen-specific antibodies. Cytotoxic T lymphocytes (CTLs) bind antigens presented by antigen presenting cells (APCs), including DCs and macrophages. In the presence of helper T cells and cytokines, including interleukin 2 (IL2), IFNγ, GM-CSF, and IL12, CTLs switch to the effector phase and destroy cells expressing a specific antigen [57]. Antigens are presented to T cells in the context of the major histocompatibility complex (MHC) class 1 associated with CD8+ CTLs and MHC class 2 with CD4+ helper T cells.

2.2. Immune Response in the Central Nervous System

Traditionally, the central nervous system (CNS) has been considered an immunologically privileged site [58], due to its separation from antibodies and lymphocytes by the blood brain barrier (BBB), the lack of a lymphatic system, the paucity of APCs [59], and the low expression level of MHC [60]. However, recent reports revised these considerations and revealed that the BBB is not an absolute barrier to lymphocyte tracking [61, 62]. Microglias are resident major APCs in the central nervous system, and play an important role in immune responses in the brain [63]. Goldmann *et al.* showed that the cerebrospinal fluid connects to the cervical lymph nodes, where naïve T cells can access it in a murine model [61]. Furthermore, it became clear that activated CTLs can pass through the BBB [62]. Taken together, these findings suggest that the CNS is not an immunologically privileged site in the strict sense, but rather an immunologically distinct organ.

2.3. Mechanism of Glioma Cell Immunoresistance

Glioma immunotherapy primes the patient's own immune system to attack glioma cells and seems to be a promising new therapy. However, clinical trials of glioma immunotherapy have yet to yield a satisfactory outcome. One reason is that GBM cells may use the mechanism of immunosuppression. GBM cells and BTSCs secrete immunosuppressive factors, and this leads to the degradation of cellular immunity and to abnormal immune cell activation [64]. These cells prevent mature DCs from functioning as APCs through the secretion of immunosuppressive cytokines, including TGF-$\beta\beta$, prostaglandin E2 (PGE2), VEGF, IL6, and IL-10 [65, 66]. TGF-$\beta\beta$ is known to expand the pool of immunosuppressive regulatory T cells, resulting in the suppression of T-cell proliferation [67]. PGE2 downregulate MHC class 2 expression and antigen processing [64]. VEGF, IL6, and IL12 activate signal transducer and activator of transcription 3 (STAT3), which has been shown to inhibit macrophage activation, induce immunosuppressive macrophage phenotype (M2), and promote GBM tumorigenesis [68-70]. Blockage of STAT3 signaling has been reported to inhibit T-cell apoptosis and reduce the BTSC-induced regulatory T cells pool [67]. BTSCs express MHC class 1 and B7-H1 [67]. The decreased expression of B7 protein prevents T-cell proliferation, and the increased expression of B7-H1 and ligand for apoptosis stimulating fragment (FasL) induces T-cell anergy and apoptosis. Thus, BTSCs have been shown to maintain the immunosuppressive environment called the immune niche [71].

2.4. Current Immunotherapy in Glioma

Various strategies of immunotherapy targeting GBM have been developed, such as cytokine therapy, adoptive immunotherapy, and active immunotherapy (Table **2**).

2.4.1. Cytokine Modulation

Cytokine modulation is a therapeutic method targeted at tumor-induced immune suppression. Moreover, in other tumors such as those of renal cancer, metastatic melanoma, and non-Hodgkin lymphoma, IL2 treatment has had positive results [72-74]. Clinical trials using cytokine modulation, including TGF-$\beta\beta$, IL2, IL12, and interferons (INF-$\alpha\alpha$, INF-$\beta\beta$, and IFN-$\gamma\gamma$), have been performed for gliomas. However, the results of these clinical trials were generally disappointing [75-80]. In contrast, among these studies, the blockage of TGF-$\beta\beta$ showed promising results in a phase IIB clinical trial [81].

Table 2:   Current Immunotherapy for Malignant Gliomas

Strategy for immunotherapy		Advantages	Disadvantages
Cytokine therapy		Relative ease of preparation and maintenance Uniform quality	Low tumor-specific cytotoxity
Adoptive immunotherapy	CTLs	High tumor-specific cytotoxity	Difficulty of preparation and maintenance
Active immunotherapy (vaccine therapy)	ATCs	Adoptable for a broad range of tumor antigens	Low antigenicity
	Peptides	Relative ease of preparation and maintenance Uniform quality	HLA restriction Restrictions of tumor antigens

CTL, cytotoxic T lymphocyte; ATC, autologous tumor cell.

Trabedersen, a TGF-$\beta\beta2$ antisense oligonucleotide, is currently in the phase III stage of clinical trials for anaplastic astrocytomas [82].

2.4.2. Adoptive Immunotherapy

Adoptive immunotherapy refers to cell transfer therapy. The patient's immune cells are matured *ex vivo* to generate activity specific for glioma cell antigens and are subsequently reinfused into the patient. The activated cells are injected into the tumor cavity or administered systemically. The first type of immune cells used in this therapy were lymphocyte-activated killer cells (LAK cells), which are autologous peripheral blood lymphocytes stimulated with IL2 *in vitro*. While the result of infusing LAK cells directly into the tumor cavity was rather encouraging [83], these cells failed to effectively migrate to the brain because of the BBB [84]. CTLs are also used for adoptive immunotherapy. CTLs are generated *ex vivo* by stimulation of peripheral blood mononuclear cells (PBMCs), which have a specific activation for tumor antigens. Activated T cells can migrate to the brain *via* the BBB and selectively affect tumor cells. Adoptive therapy combined with other strategies of immunotherapy has shown to be promising. Genetically, modified T cells that express a chimerical antigen receptor for tumor antigens, such as IL-13, Receptor $\alpha2$ (IL-13R$\alpha2$), and HER2 have been reported to induce GBM regression in animal models [85, 86].

2.4.3. Active Immunotherapy

Active immunotherapy is otherwise known as vaccine therapy. Vaccines are derived from autologous tumor cells, tumor cell lysates, or specific peptides that encode tumor antigens. Identification of a large number of tumor associated antigens (TAAs) has advanced cancer vaccines, as in 1991, it became technically possible to amplify TAAs by cDNA cloning of these TAAs [87]. An injection of TAAs stimulates APCs to present tumor antigens to the patient's immune system. Then, lymphocytes sensitized against tumor antigens migrate to the tumor and attack the tumor cells [88]. Vaccines seem to be promising in inducing an anti-glioma immune response without major side effects. Some of the approaches for active immunotherapy involve using peptides, DCs, autologous tumor cells, and heat shock proteins (HSPs). Peptide vaccines are discussed in detail later.

DCs are the main APCs that activate an immune response. DCs pulsed with tumor antigens are injected into patients as vaccine. Many animal and clinical studies have been conducted using DC-based vaccination. The most recent results of phase I/II trial for 77 patients with newly diagnosed GBM confirmed that the treatment was feasible without major toxicity and the median overall survival was 18.3 months [89].

Vaccines using autologous tumor cells, cultured and modified from the resected original tumors, activate the immune system with an increased number of potential glioma antigens when they are injected back into the patients. Recent clinical trials for GBM support the safety of this method, although the results are controversial. One report could not show any significant effect [90], while another demonstrated a prolonged median survival [91]. In a pilot study using autologous formalin-fixed tumor vaccines, performed by Ishikawa *et al.* the safety was confirmed and vaccine therapy improved the outcome of GBM [92]. Further clinical investigations are continuing.

HSPs, instead of DCs, are used to deliver tumor antigens. HSPs are chaperone proteins that aid in the folding of many proteins within the cell. Specific HSPs that have been isolated from the excised tumors and purified, are used as vaccines [93]. In addition, HSPs are potent immune activators. A favorable outcome has been reported [94], and clinical trials using an HSP vaccine for GBM are in progress.

2.5. Peptide-Based Vaccines

Peptide-based vaccines are the most promising immunotherapy technique, and now, there are several ongoing clinical studies. The peptides used as cancer vaccines consist of 9 amino acids capable of binding to a particular MHC class 1 antigen with the ability to activate CTLs reactive to tumor cells. Peptide antigens are ingested and processed by APCs. Then, APCs present these peptide antigens in association with MHC class 1 molecules on their surface. Peptide antigen/MHC class 1 complexes stimulate CD8+ T cells to make antigen-specific CTLs. These activated CTLs travel from the lymph nodes into the blood circulation and finally migrate to the brain where they eliminate glioma cells (Figure **1**). Although several clinical trials of peptide-based vaccines for various cancers have been reported, the responses were not significant [95]. In contrast, some recent clinical trials of peptide-based vaccines for malignant gliomas have obtained promising results.

2.5.1. EGFRvIII

The epidermal growth factor receptor vIII (EGFRvIII) is a mutated form of the wild-type EGFR. It is not
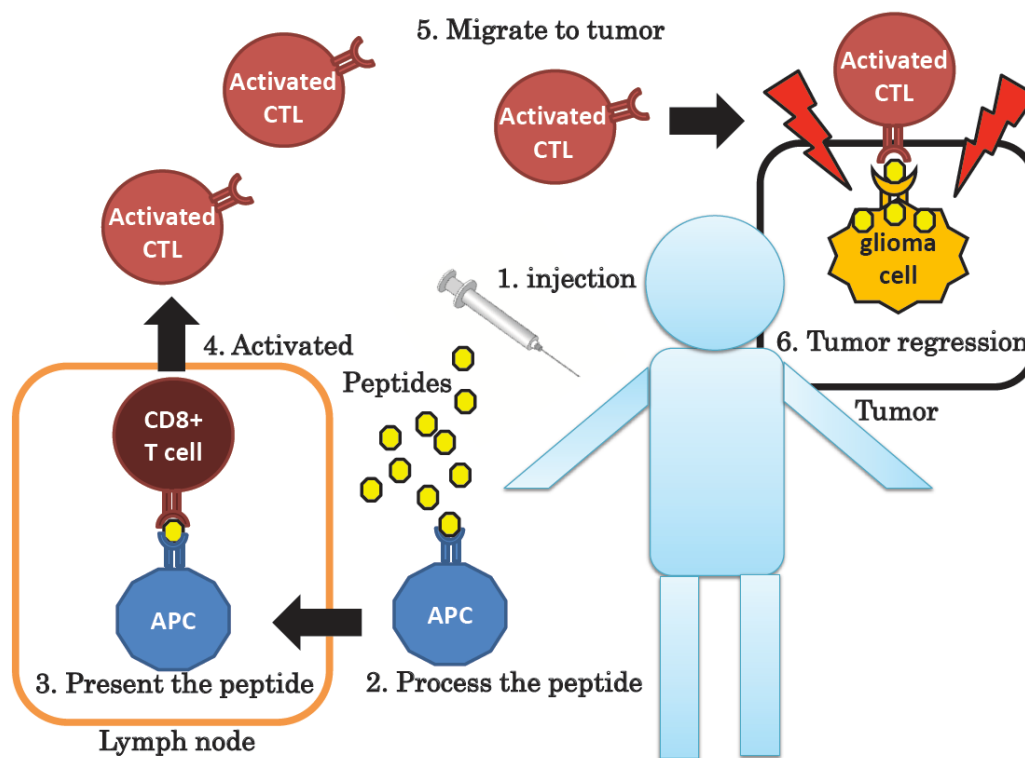
Figure 1: Peptide vaccine strategy.
Intradermal injection of peptide vaccine is repeated. Antigen-presenting cells (APCs) process the peptide antigen and then present it in association with antigen/major histocompatibility complex (MHC) class 1 molecules on the surface of APCs. Peptide antigen/MHC class 1 complexes stimulate CD8+ T cells to make antigen-specific cytotoxic T cells (CTLs) in the lymph node. Activated antigen-specific CTLs migrate to the brain and destroy glioma cells, leading to tumor regression.

expressed in the normal brain, but is overexpressed in malignant glioma and has a prevalence of 20–30% in GBMs [96, 97]. In contrast to earlier vaccine trials targeting EGFRvIII, which yielded unimpressive results, current trials using a novel EGFRvIII epitope have produced encouraging data [5, 98]. An EGFRvIII-targeted peptide vaccine began a phase I clinical trial, the Vaccine for Intra-Cranial Tumors I (VICTORI), and continued to the phase II study, A Complementary Trial of an Immunotherapy Against Tumor Specific EGFRvIII (ACTIVATE) [99]. In the VICTORI phase I trial, 20 patients with malignant glioma (WHO grade 3/4) were enrolled and 16 of these patients were administered vaccines after surgery and radiotherapy. Autologous DCs were pulsed with 500 µg of an EGFRvIII Peptide (PEPvIII, LEEKKGNYVVTDHC) conjugated to the adjuvant molecule keyhole limpet hemocyanin (KLH). All patients were vaccinated 3 times *via* an intradermal injection. The median time to progression was 11.7 months and the median survival of GBM patients treated with the vaccines was 22.8 months; both of these time periods are longer than those of patients treated with temozolomide, whose results are 6.3 months and 14.6 months, respectively [100].

ACTIVATE, the following phase II trial, evaluated the efficiency of PEPvIII-KLH and GM-CSF without the use of DCs [5]. Patients with newly diagnosed GBM were enrolled into this trial and vaccinated monthly until there was evidence of tumor progression. The median time to progression was 14.2 months and the median survival was 32 months, both of which are longer than the unvaccinated control group. In addition, 100% of recurrent tumors lost the expression of EGFRvIII. This suggests that the vaccine therapy was immunologically effective with successful elimination of EGFRvIII-positive tumor cells. The follow-up phase II multicenter trial enrolled 21 patients with EGFRvIII-positive GBMs who were treated monthly with PEPvIII-KLH, following the standard surgical, radio-, and temozolomide therapy, until tumor progression was observed. The median time to progression was 16.6 months [6]. The EGFRvIII-targeted vaccine (CDX-10) is in an ongoing phase III randomized clinical trial and awaiting results.

2.5.2. Personalized Peptide Vaccine

In personalized peptide vaccines, patients who had exhibited a preexisting response to specific peptides were vaccinated with these peptides. By utilizing this

strategy, a faster and stronger activation of CTLs can be induced [101]. The results of a phase I study of personalized peptide vaccination for malignant glioma have been reported [7]. The study enrolled 25 malignant glioma patients. Prevaccination PBMCs and plasma were collected to examine the response to peptides in HLA-A24+ or HLA-A2+ patients. The most frequently reactive peptides were derived from squamous cell carcinoma antigen recognized by T cells 3 (SART3), lymphocyte-specific protein tyrosine kinase, and multidrug resistance-associated protein 3 (MRP3) antigens. The clinical response induced 5 partial responses (PR) and the median survival was 18 months. A recent clinical study of personalized peptide vaccines for HLA-A24+ patients with GBM also reported promising results [8]

2.5.3. WT1

The WT1 gene was isolated as a gene responsible for Wilms tumor, and it is involved in cell proliferation and apoptosis [102]. As the WT1 gene was frequently overexpressed in various tumors including gliomas, the WT1 protein was an attractive target for immunotherapy. A phase II clinical trial for a WT1 peptide vaccination for GBM was reported in 2008 [9]. Twenty-one patients with recurrent GBM were enrolled, and the HLA-A2402-restricted modified 9-mer WT1 peptide was used. The clinical response induced 2 PRs and the median progression-free survival was 5 months. Several clinical studies are now ongoing.

2.5.4. IL-13 Receptor α2

IL-13Rα2 is a cell-surface receptor highly expressed on glioma cells, but not at significant levels in normal brain tissue. Therefore, the IL-13Rα2 antigen is thought to be a good target for immunotherapy [103]. IL-13Rα2 vaccine has been demonstrated to induce strong immunological antitumor effects in a mouse glioma model [104, 105]. Iwami *et al.* conducted a phase I trial of DC vaccination in patients with recurrent malignant glioma using 2 IL-13Rα2-derived peptides restricted to HLA-A*0201 and HLA-A*2402 [10]. Eight patients were enrolled, 1 achieved stable disease for 16 months and another had 1 lesion that dramatically regressed for 4 months.

3. TARGETING BTSCs USING IMMUNOTHERAPY

BTSCs are resistant to conventional therapies including chemotherapy and radiotherapy due to the expression of several ABC transporters, an active DNA-repair capacity, and resistance to apoptosis [33,

106]. In addition, BTSCs create their own immune niche, which contributes to the mechanism of immune privilege as described above. Thus, BTSCs and their immune niche are potential therapeutic targets. Several recent studies reported immunotherapy that targeted BTSCs [11-13]. Pellegatta *et al.* found that DC immunotherapy could target murine glioma stem-like cells—GL261 neurospheres [11]. In this study, DC loaded with GL261 neurospheres eliminated 60% of GL261 neurosphere tumors, whereas DC loaded with GL261-adherent cells had no effect on theGL261 neurosphere tumors. Similar results pertaining to GBM neurosphere-targeting immunotherapy have been reported elsewhere [12]. Xu *et al.* demonstrated that an antigen-specific response could be generated from DC vaccination using rat glioma stem-like cell-associated antigens. SOX2 was regarded as the stem cell associated-antigen as it is highly expressed on glioma stem-like cells. Specific CTLs were raised against HLA-A0201-restricted SOX2-derived peptides that were capable of lysing glioma cells [107]. Ueda *et al.* demonstrated that vaccination with SOX6 DNA induced CTLs specific for glioma in mice [108]. *In vitro* stimulation with HLA-A*0201 (A2)- and HLA-A*2402 (A24)-restricted SOX6 peptides resulted in the induction of peptide-specific CTLs in PBMCs derived from glioma patients. These CTLs were able to lyse the majority of glioma cell lines and BTSC lines derived from human GBM [13]. These results suggest that vaccine immunotherapy may have the potential to deplete human BTSCs.

CONCLUSION

Although not yet fully characterized, BTSCs are increasingly being recognized as a main cause of resistance to treatment in malignant brain tumors, especially GBM. Consequently, it is imperative that these cells are targeted using novel and specific therapies. Recent advances in immunotherapy mean that it is possible to target BTSCs and to tailor the therapy to the characteristics of individual patients. Immunotherapy can be considered a promising new treatment modality, which, perhaps in combination with surgery and chemotherapy, might prolong survival for patients with GBM.

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