The role of vascular endothelial growth factor in the hypoxic and immunosuppressive tumor microenvironment: perspectives for therapeutic implications

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Abstract

The microvasculature and immune cells are major components of the tumor microenvironment (TME). Hypoxia plays a pivotal role in the TME through hypoxia-inducible factor 1- α (HIF-1 α) which upregulates vascular endothelial growth factor (VEGF). VEGF, an angiogenesis stimulator, suppresses tumor immunity by inhibiting the maturation of dendritic cells, and induces immunosuppressive cells such as regulatory T cells, tumor-associated macrophages, and myeloid-derived suppressor cells. HIF-1 α directly induces immune checkpoint molecules. VEGF/VEGF receptor (VEGFR)-targeted therapy as a cancer treatment has not only anti-angiogenic effects, but also immune-supportive effects. Anti-angiogenic therapy has the potential to change the immunological “cold tumors” into the “hot tumors”. Glioblastoma (GB) is a hypervascular tumor with high VEGF expression which leads to development of an immuno suppressive TME. Therefore, in the last decade, several combination immunotherapies with anti-angiogenic agents have been developed for numerous tumors including GBs. In particular, combination therapy with an immune checkpoint inhibitor and VEGF/VEGFR-targeted therapy has been suggested as a synergic treatment strategy that may show favorable changes in the TME. In this article, we discuss the cross talk among immunosuppressive cells exposed to VEGF in the hypoxic TME of GBs. Current efficient combination strategies using VEGF/VEGFR-targeted therapy are reviewed and proposed as novel cancer treatments.

Keywords VEGF · Hypoxia · Regulatory T cell · Tumor-associated macrophage · Myeloid-derived suppressor cell · Immune checkpoint molecule · Tumor microenvironment

Introduction

The tumor microenvironment (TME) consists of immune cells, fibroblasts, endothelial cells, extracellular matrix, and some signaling molecules such as chemokines. The TME shows an immunosuppressive effect and plays critical roles in tumor growth, angiogenesis, and metastasis [1].

Glioblastomas (GBs) are the most aggressive and vascularized primary brain tumors [2]. Despite multimodal therapy including surgical removal, radiation, and chemotherapy, GBs are essentially incurable [3, 4]. The heterogeneity, infiltrative characteristics, presence of glioma stem cells, and function of blood-brain barrier have been appointed as the main causes of therapeutic resistance and malignant relapse [3, 4]. Furthermore, the lack of anti-tumor immune response due to an immunosuppressive TME also contributes to the treatment failure. Immune checkpoint molecules, exhaustion of cytotoxic T lymphocytes (CTLs), and immunosuppressive cells in hypoxic conditions induce the immunosuppressive TME. Tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs) are major components of the immunosuppressive cells in the TME of GBs [5]. Programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) are immune checkpoint molecules that are associated with immunosuppressive cells in the TME. PD-L1 expressed on tumor cells

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binds to PD-1 expressed on activated T cells and negatively regulates immune responses [6, 7].

Vascular endothelial growth factor (VEGF) is a key mediator of tumor angiogenesis and a major target for anti-angiogenic therapy for various malignant tumors including GBs [2, 8, 9]. VEGF is induced by hypoxia through a hypoxia-inducible factor 1 alpha (HIF-1 α)-depending pathway, which contributes to immune suppression in the TME [10]. Therefore, anti-VEGF (bevacizumab; Bev) or VEGF receptor (VEGFR)-targeted (sunitinib, sorafenib) agents as a cancer treatment induce not only anti-angiogenic effects, but also immune-supportive effects [11–14]. Recently, the significance of the PD-1/PD-L1 immune checkpoint system has received attention in several types of tumors [6, 7]. Anti-PD-1 and PD-L1 antibodies exert a potent effect in inhibiting tumor growth in melanomas, non-small lung cancer, and kidney cancer [15]. However, immunologically “cold” tumors including GBs did not have advantages by immune checkpoint inhibitors [16]. Anti-angiogenic therapy may convert immunologically “cold” tumors to “hot” tumors. Therefore, combination usage of an anti-angiogenic agent and an immune checkpoint inhibitor may be a strategy to overcome the mechanism of resistance of immunotherapies for “cold” tumors [17]. Over the last decade, several anti-angiogenic therapies combined with chemotherapies and immunotherapies have been developed for elimination of the immunosuppressive TME. For further development of these strategies, we need to understand alterations in the TME following chemotherapies and immunotherapies with or without anti-VEGF/VEGFR therapy.

In this article, we discuss the cross talk among immunosuppressive cells exposed to VEGF in the hypoxic TME of GBs. The current efficient combination strategies using VEGF/VEGFR-targeted therapy are reviewed. Finally, alterations in the TME following these combination strategies are also summarized to understand the mechanisms of action and resistance, followed by a proposal for novel cancer therapies.

VEGF

VEGF plays an important role in vascular development and in diseases involving abnormal growth of blood vessels. Since discovery of its dual roles in endothelial proliferation and vascular permeability [18, 19], VEGF has been considered a key mediator of neovascularization in tumors (Fig. 1). Elevated VEGF levels are associated with poor clinical outcomes in numerous tumors including GBs [2, 20]. In addition to angiogenic effects, VEGF suppresses the anti-tumor immune response [15]. VEGF inhibits maturation of dendritic cells (DCs), resulting in inactivation of CTLs [21]. VEGF also induces an immunosuppressive TME by strongly inducing Tregs, TAMs, and MDSCs [22].

Furthermore, VEGF enhances the expression of PD-1 on CD8⁺ CTLs and Tregs in a VEGFR2-dependent manner (Fig. 1) [23]. Tumor-derived VEGF, interleukin (IL)-10, and prostaglandin E3 cooperatively induced Fas ligand expression in endothelial cells, leading to exhaustion of CTL but not Tregs (Fig. 1) [24].

VEGF is induced by hypoxia via activation of the transcription factor, HIF-1, which could play an important role in triggering tumor angiogenesis [25, 26]. The TME is mainly altered by hypoxia and acidosis [27]. Hypoxia supports the escape of tumor cells from immune surveillance by recruiting TAMs, Tregs, and MDSCs into the TME directly or through upregulation of VEGF [28].

TAMs

Macrophages often behave as immunosuppressive cells and contribute to inflammatory diseases. Macrophages express different functional programs in response to microenvironmental signals, defined as M1/M2 polarization [29]. M2 macrophages produce growth factors and anti-inflammatory cytokines to suppress the host immune response, resulting in tumor progression [29].

TAMs typically behave as M2 macrophages [30], playing a pivotal role in the TME [28, 30]. The interaction between the tumor cells and TAMs is promoted via macrophage colony-stimulating factor and its receptor [29]. TAMs induce various growth factors such as basic fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, and platelet-derived growth factor [29]. In addition, the lack of arginine by arginase I, and IL-10, transforming growth factor (TGF)- β , and prostaglandin F2 produced by TAMs suppress effector T cells [29]. TAMs produce VEGF and matrix metalloproteinase 9, which promote angiogenesis, invasion, and metastasis [29]. Although the existence of TAMs is still controversial as a prognostic biomarker for cancer patients, most reports have demonstrated that increased TAMs are related to poor prognosis in GBs; breast, esophagus, and liver cancers; and malignant lymphoma [31]. Interestingly, TAMs infiltration is associated with the resistance to anti-angiogenic therapy through downregulation of macrophage migration inhibitory factor in GBs [32]. VEGF in the hypoxic TME is a key factor for transitioning from the M1 to M2 macrophage phenotype [33]. Furthermore, HIF-1 α promotes the migration and differentiation of TAMs from immature myeloid cells via VEGF exposure [17].

Tregs

Tregs (CD4 + CD25 + Foxp3⁺) play an active and significant role in the progression of tumors, and play an important role in suppressing tumor-specific immunity [34, 35]. Tregs suppress T cell-mediated immune responses

Fig. 1 Role of VEGF in the tumor microenvironment. VEGF has dual effects of vascular biological and tumor immunological regulation in the tumor microenvironment. VEGF plays a pivotal role in inducing vacular endothelial cells and immunosuppressive cells in hypoxic conditions. *DCs* dendritic cells, *ECs* endothelial cells, *IFP*

interstitial fluid pressure, *MDSCs* myeloid-derived suppressor cells, *PD-1* programmed death-1, *PD-L1* programmed death ligand 1, *Tregs* regulatory T cells, *TAMs* tumor-associated macrophages, *TILs* tumor-infiltrating lymphocytes, *VEGFR* vascular endothelial growth factor receptor, *VVO* vesiculo-vacuolar organelle

via TGF- β , IL-10, and IL-35. Tregs induce the apoptosis of effector T cells through granzyme B. Consumption of IL-2 by Tregs inhibits effector T cells proliferation. Furthermore, Tregs directly suppress DCs [35]. Increased Tregs in tumor tissue or peripheral blood are significantly associated with shorter survival in most cancer patients including those with GBs [36]. Tregs may be critical for evaluation of the clinical significance of immunosuppressive effects after anti-angiogenic therapy. To detect Tregs more precisely, effector Tregs are defined as CD45RA-Foxp3^{high}CD4⁺ which may reflect an immune suppressive function. Non Tregs (CD45RA-Foxp3^{low}CD4⁺), which produce inflammatory cytokines such as interferon- γ , are included in the CD4⁺CD25⁺Foxp3⁺ cell population [36].

The role of HIF-1 α has also been implicated in direct regulation of the differentiation of Tregs and in promotion of the recruitment of Tregs to the TME via overexpression of CC chemokine ligand 22 and 28 [37]. HIF-1 α induces VEGFR2-expressing Tregs through VEGF production [37].

MDSCs

MDSCs are a type of myeloid cells that can differentiate into macrophages, DCs and granulocytes, thus suppressing CTLs. In humans, MDSCs are CD11b⁺CD33⁺Cd14⁺HLA-DR⁻ [38]. CD115, CD124, and VEGFR were also identified in MDSCs [39]. MDSCs are induced by IL-6, VEGF, and prostaglandin E2, which are produced by tumor cells. In addition, MDSCs are activated by interferon- γ , IL-4, IL-13, and TGF- β produced by CTLs and tumor stromal cells [38]. MDSCs also produce TGF- β , IL-10, and metalloproteinase 9 [40]. Interestingly, recent findings suggest that natural killer T cells activated by α -GalCer-loaded CD11b⁺Gr1⁺MDSC can acquire the ability to convert immunosuppressive MDSCs into immunity-promoting antigen-presenting cells. Reprogramed MDSCs show upregulation of expression of CD11b, CD11c, and CD86, which support immunity by antigen-specific CTLs without increasing Tregs [22, 41]. GB patients with a more favorable prognosis exhibit decreased MDSCs and increased DCs compared

to those with a worse prognosis [42]. Interactions between MDSCs and glioma stem cells via migration inhibitory factor enhances the function of MDSCs, which could be targeted to reduce the growth of GBs [42]. In addition, MDSCs directly promotes angiogenesis, which is associated with refractoriness to anti-angiogenic therapy [43]. VEGF is strongly associated with MDSC accumulation in GBs [44, 45]. HIF-1 α has also been implicated in direct regulation of the function and differentiation of MDSCs in the hypoxic TME [46].

PD-1/PD-L1

PD-1 is expressed on CD8+T cells and Tregs. PD-L1 is expressed on tumor cells in numerous malignant tumors including GBs and binds to PD-1 to negatively regulate the immune response of CD8+T cells [6, 7]. A recent study showed that PD-1 is expressed on TAMs and correlates negatively with phagocytic potency, demonstrating its relevance to tumor immunity [47]. PD-L1 protein expression was identified in 61 to 88% of patients with GBs [48]. PD-1/PD-L1 expression is associated with poor prognosis for patients with GBs [49, 50]. Garber et al. demonstrated that GB specimens have a higher frequency of PD-1+ tumor-infiltrating lymphocytes (TILs) compared with lower-grade gliomas, whereas PD-L1 expression does not significantly differ among malignant grades [51]. Isocitrate dehydrogenase (IDH)-wild-typed GBs are more “immunologically active” than IDH-mutated GBs. GBs with wild-type IDH display a higher number of TILs and elevated expression of PD-L1 compared with IDH-mutant GBs. Therefore, IDH-wild-type GBs may be more readily targeted by PD-1/PD-L1 checkpoint blockade [52]. In particular, PD-L1 is strongly expressed in the mesenchymal subgroup of GBs [53].

A previous study demonstrated that PD-1 expression on CD8+T cells and Tregs is induced by VEGF [23]. Similarly, PD-L1 is upregulated via VEGF exposure in some types of tumors including GBs [53]. Immunologically “cold” tumors including GBs are not good targets for immune checkpoint inhibitors [16]. Anti-angiogenic therapy has the possibility to change “cold” tumors into “hot” tumors with a favorable microenvironment. Therefore, combination therapy with an immune checkpoint inhibitor and VEGF-targeted therapy is expected to be an efficient treatment strategy [17]. In addition, hypoxia directly causes upregulation of PD-L1 and CTLA-4 on MDSCs, TAMs, DCs, and tumor cells through HIF-1 α [54]. HIF-2 α is also associated with PD-L1 expression on tumor cells in metastatic renal cell carcinoma [55]. Blocking HIF-1 α by agonizing nitric oxide signaling decreased PD-L1 expression and promotes CTL-mediated lysis [56].

Cross talk in the TME

VEGF promotes the accumulation of TAMs, Tregs, and MDSCs in tumor tissue and secondary lymphoid organs [10]. In addition, HIF-1 α activation is also a major component of the hypoxic TME. HIF-1 α upregulation directly drives recruitment of immature myeloid cells and Tregs, and fosters their phenotypic conversion into highly suppressive MDSCs and TAMs [58]. Recent studies demonstrated functional cross talk among TAMs, Tregs, and MDSCs that was strongly associated with hypoxia-induced VEGF production [43, 57, 59]. Tregs modify the phenotype of TAMs to express inhibitory B7-H molecules. Tregs depletion significantly downregulates the expression of immune suppressive molecules such as B7-H1 on MDSCs and TAMs, and also reduces tumor growth. In addition, Tregs produce IL-10, IL-4, and IL-13, and induce monocyte differentiation toward TAMs [43, 57, 59]. MDSCs are reported to differentiate into TAMs in the hypoxic microenvironment, which is regulated by STAT3 activity [60]. Since these immunosuppressive cells and immune checkpoint molecules show cross talk in the hypoxic TME, anti-VEGF/VEGFR therapy can induce tumor oxygenation [61], resulting in an immune-supportive TME.

Alteration in the TME following chemotherapy with or without anti-VEGF/VEGFR therapy

Alteration of immunosuppressive cells and immune checkpoint molecules has been investigated utilizing clinical samples before and after chemotherapy [36, 49, 62–103], but results have been inconsistent (Tables 1 and 2) [36, 49, 62–103]. Metronomic cyclophosphamide decreases Tregs [62, 66–68, 83], and gemcitabine decreases both Tregs and MDSCs [35, 65, 103]. Tregs and MDSCs are not increased when chemotherapeutic agents are co-administered with immunomodulatory agents such as vaccination and IL-2 (Table 1) [49, 63, 75, 78]. However, most chemotherapeutic agents including temozolomide tend to increase Tregs and MDSCs [71, 72, 74, 79, 84]. In addition, chemotherapy also enhances PD-1/PD-L1 via TGF- β induced epithelial-mesenchymal transition [69].

In contrast, combinational chemotherapy with VEGF/VEGFR-targeted agents could make the TME immune supportive [23, 96]. VEGF/VEGFR-targeted therapy such as Bev, sorafenib, and sunitinib reduces the population of Tregs in the peripheral blood and tumor tissue in GBs, metastatic colorectal cancer, hepatocellular carcinoma, and renal cell carcinoma (Table 2) [86–103]. However, some studies using paired peripheral blood before and after VEGFR-targeted therapy demonstrated that Tregs and PD-1 were significantly increased [104]. The effect of anti-angiogenic therapy has been highly controversial regarding MDSCs [91, 105].

Table 1 Alteration in immunosuppressive cells and immune checkpoint molecules following chemotherapy without anti-VEGF therapy

Author, year	Type of tumor	Anti-angiogenic agent	Assay	Sample	PD-1	PD-L1	Tregs	TAMs	MDSCs	Clinical outcome
Ghiringhelli F, 2007	CRC, RCC, GC	CTX	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	N/A
Greten TF, 2010	HCC	CTX	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	N/A
Fadul CE, 2011	GBM	TMZ	FACS	PBMCs	N/A	N/A	N/A	Up	N/A	N/A
Mitchell DA, 2011	GBM	TMZ/anti-IL-2R antibody	FACS	PBMCs	N/A	N/A	Anti-IL-2R(-): up Anti-IL-2R(+): down	N/A	N/A	N/A
Sampson JH, 2012	GBM	TMZ/EGFRvIII targeted vaccine/anti-IL2R antibody	FACS	PBMCs	N/A	N/A	Anti-IL-2R(-): up Anti-IL-2R(+): down	N/A	NC	N/A
Ellebaek E, 2012	Melanoma	CTX/IL-2/DC vaccine	FACS	PBMCs	N/A	N/A	Up	N/A	N/A	Tregs/MDSCs; not correlated with clinical outcome
Ge Y, 2012	Breast cancer	CTX	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	Tregs: not correlated with clinical outcome
Verma C, 2013	Breast cancer	CTX, ADM, DTX, CAPE	FACS	PBMCs	N/A	N/A	Down	N/A	Down	N/A
Tarhini AA, 2014	Melanoma	Ipilimumab	FACS, IHC	PBMCs, Tissue	N/A	N/A	Up	N/A	Down	<ul style="list-style-type: none"> Decrease MDSCs; improve OS Increase Tregs; improve PFS
Shinto E., 2014	Rectal cancer	UFT, radiation	IHC	Tissue	N/A	N/A	NC	N/A	N/A	CD8/Foxp3 ratio: not significant
Koumariou A, 2014	Breast, lung, Ovary, Prostate cancer, CRC	Epirubicin/PTX/CBCDA/Vinorelbine/Capecitabine/TMZ	FACS	PBMCs	N/A	N/A	Up	N/A	N/A	N/A
Homma Y, 2014	Pancreatic cancer	GEM	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	Tregs: not correlated with clinical outcome
Annels NE, 2014	Pancreatic cancer	GEM-CAPE-GV1001(vaccine)	FACS	PBMCs	N/A	N/A	N/A	N/A	GV (-) up or down, GV(+) down	N/A
Teng F, 2015	Rectal cancer	Neoadjuvant chemoradiation	IHC	Tissue	N/A	N/A	NC	N/A	NC	<ul style="list-style-type: none"> Low MDSCs; sensitive to CRT Tregs: not associated with tumor response

Table 1 (continued)

Author, year	Type of tumor	Anti-angiogenic agent	Assay	Sample	PD-1	PD-L1	Tregs	TAMs	MDSCs	Clinical outcome
Takahashi H, 2016	Head and Neck SCC	DTX-CDDP-5FU	FACS	PBMCs	N/A	N/A	Up and down	N/A	Up and down	N/A
Eriksson E, 2016	Pancreatic cancer	GEM	FACS	PBMCs	N/A	N/A	Down	N/A	Down	N/A
Batich KA, 2017	GBM	TMZ/pp65-targeted vaccine	FACS	PBMCs	N/A	N/A	Up	N/A	N/A	N/A
Miyazaki T, 2017	GBM	TMZ/AFTV vaccine	IHC	Tissue	AFTV(-): up or down AFTV(+): up	N/A	NC	N/A	N/A	• High PD-1: favorable PFS low PD-1: improve OS
Plekanou V, 2017	Breast cancer	CTX, DOX	IHC	Tissue	N/A	Down	N/A	N/A	N/A	N/A
Wesolowski R, 2017	Breast cancer	PTX, anti-HER2 antibody	FACS	PBMCs	N/A	N/A	N/A	N/A	Up	N/A
Haratake N, 2017	Lung cancer	DTX/PTX-nivolumab	IHC	Tissue	N/A	Up	N/A	N/A	N/A	N/A
Mesnage SJL, 2017	Ovarian cancer	CBDCA/PTX	IHC	Tissue	N/A	Up or down	N/A	N/A	N/A	N/A
Liang Y, 2018	Uterus cervical cancer	CDDP/PTX	IHC	Tissue	N/A	N/A	Down	N/A	N/A	CD8/Foxp3 high: favorable outcome
Rojko L, 2018	Lung cancer	CDDP and GEM, CBDCA and PTX	IHC	Tissue	Up and down	down	N/A	N/A	N/A	N/A
Kim HS, 2018	Ovarian cancer	CBDCA/PTX	IHC	Tissue	N/A	Up or down	N/A	N/A	N/A	Tregs/PD-L1; not correlated with clinical outcome

ADM adriamycin, *AFTV* autologous formalin fixed tumor vaccine, *CBDCA* carboplatin, *CDDP* cisplatin, *CRT* chemoradiation, *CTX* cyclophosphamide, *DOX* doxorubicin, *DTX* docetaxel, *EGFR* epidermal growth factor receptor, *FACS* fluorescence-activated cell sorting, *FU* fluorouracil, *GBM* glioblastoma, *GEM* gemcitabine, *GV* gemcitabine and vinorelbine combination, *HCC* hepatocellular carcinoma, *IHC* immunohistochemistry, *IL-2R* interleukin-2 receptor, *MDSCs* myeloid-derived suppressor cells, *n.a.* not analyzed, *nd* not described, *NSCLC* non-small cell lung cancer, *OS* overall survival, *PBMCs* peripheral blood mononuclear cells, *PD-1* programmed death-1, *PD-L1* programmed death ligand 1, *PFS* progression free survival, *PR* partial response, *PTX* paclitaxel, *SCC* squamous cell carcinoma, *Tregs* regulatory T cells, *TAMs* tumor-associated macrophages, *TMZ* temozolomide, *UFT* uracil and tegafur

Table 2 Alteration of immunosuppressive cells and immune checkpoint molecules following anti-VEGF therapy

Author, year	Type of tumor	Anti-angiogenic agent	Assay	Sample	PD-1	PD-L1	Tregs	TAMs	MDSCs	Clinical outcome
Adotevi O, 2010	RCC	Sunitinib	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	N/A
Desar IM, 2011	RCC	Sunitinib	FACS, IHC	PBMCs, Tissue	N/A	N/A	Down	N/A	N/A	N/A
Vizlo B, 2012	Pancreatic cancer	GEM, OX, CAPE, Bev	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	N/A
Lu-Emerson C, 2013	GBM	Bev, Cediranib, Cabozantinib, Vatalanib, Thalidomide	FACS, IHC	PBMCs, Tissue	N/A	N/A	N/A	Bev(-): no change Bev(+): up	N/A	N/A
Terme M, 2013	CRC	Sunitinib, Mastinib	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	N/A
Hodi FS, 2014	Melanoma	Bev, Ipilimumab	FACS, IHC	PBMCs, Tissue	N/A	N/A	NC	Up	N/A	N/A
Donini M, 2015	RCC	5FU/GEM/IL-2/IFN- α -Bev	FACS	PBMCs	N/A	N/A	Bev(-): up Bev(+): down	N/A	N/A	Decreased Tregs: PR or CR
Wallin JJ, 2016	RCC	Bev, Atezolizumab	FACS, IHC	PBMCs, Tissue	N/A	Up	N/A	Up	N/A	N/A
Liu XD, 2016	RCC	Bev, sunitinib	IHC	Tissue	N/A	Up	Up	N/A	N/A	N/A
Kalathil SG, 2016	HCC	Sunitinib	FACS	PBMCs	N/A	N/A	Down	N/A	Down	Decreased PD1/ decreased Tregs: favorable outcome
Feng PH, 2016	Lung cancer	gefitinib/erlotinib-Bev	FACS	PBMCs	N/A	N/A	N/A	N/A	Down	Low MDSCs: favorable outcome
Koinis F, 2016	Lung cancer	taxane/PEM/GEM/ VIN-Bev	FACS	PBMCs	N/A	N/A	N/A	N/A	Down	MDSCs: no correlation with clinical outcome
Limagne E, 2016	Colorectal cancer	FOLFOX-Bev	FACS	PBMCs	N/A	N/A	Up and down	N/A	Up and down	Decreased G-MDSCs: favorable outcome
Du Four S, 2016	GBM (rec)	CCNU-Axitinib	FACS	PBMCs	Up	N/A	CCNU(-): up CCNU(+): up	N/A	NC	N/A
Thomas AA, 2017	GBM	Bev	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	N/A
Finke JH, 2018	RCC	Sunitinib	FACS	PBMCs	N/A	N/A	Down	N/A	N/A	N/A
Tada Y, 2018	GC	Ramucirumab	FACS, IHC	PBMCs, Tissue	N/A	N/A	Down	N/A	N/A	Reduction of Tregs/ PD-1: favorable outcome
Quillien V, 2019	GBM	Bev	FACS	PBMCs	N/A	N/A	Down	N/A	NC	Decreased neutrophils/ decreased Tregs: favorable outcome
Tamma R, 2019	RCC	Bev	IHC	Tissue	N/A	N/A	N/A	NC (CD68: down)	N/A	Decreased CD68: favorable outcome
Tamura R, 2019	GBM	RT/TMZ-Bev	IHC	Tissue	Down	Up and down	Down	Down	N/A	N/A

Bev bevacizumab, CAPE capecitabine, CCNU lomustine, CR complete response, FACS fluorescence-activated cell sorting, FU fluorouracil, GBM glioblastoma, GC gastric cancer, GEM gemcitabine, G-MDSC Granulocyte-like myeloid-derived suppressor cells, HCC hepatocellular carcinoma, IFN- α interferon-alpha, IHC immunohistochemistry, MDSCs myeloid-derived suppressor cells, n.a. not analyzed, n.d. not described, NC not changed, OX oxaliplatin, PBMCs peripheral blood mononuclear cells, PEM pemetrexed, PD-1 programmed death ligand 1, PD-L1 programmed death ligand 1, RT radiotherapy, PR partial response, RCC renal cell carcinoma, Treg regulatory T cells, TAMs tumor-associated macrophages, TMZ temozolomide, VIN vinorelbine

Serum VEGF-A levels are correlated with the population of MDSCs, and Bev may decrease MDSCs [90, 94, 95]. Sunitinib and sorafenib may also decrease the population of MDSCs and recover the Th1 reaction in the patients of hepatocellular carcinoma and metastatic kidney cancer [93, 105]. However, the population of MDSCs does not change following Bev and axitinib, despite decreasing in serum VEGF of patients with kidney cancer [106]. The status of MDSCs following anti-VEGF/VEGFR therapy is inconsistent [88, 90, 93–95, 97, 98, 104].

Furthermore, in the recurrent stage after anti-angiogenic therapy, the status of immunosuppressive cells and immune checkpoint molecules is also highly controversial (Table 2). VEGF-targeted therapy can lead to either tumor oxygenation or tumor hypoxia [14, 61, 107]. Therefore, both an immune-supportive TME in normoxic conditions and a hypoxia-induced immunosuppressive TME have been reported after VEGF-targeted therapy [14, 61, 107]. Tregs in the peripheral blood are increased in patients with recurrent GBs after development of resistance to VEGFR inhibitors [89]. An increased level of PD-1 expression on CD4 and CD8 T cells was reported in patients with GBs or metastatic renal cell carcinoma that is refractory to VEGFR-targeted therapy [89, 109]. Previous studies have demonstrated changes in the TME using tumor specimens resected under and after Bev therapy. Bev downregulates the expression of PD-1 and PD-L1 immune checkpoint molecules, suppresses the infiltration of TAMs and Tregs, and increases CTL infiltration. Importantly, the conditions are sustained during long-term Bev usage [14]. VEGF persistently contributes to tumor growth, even if secondary signaling pathways are upregulated. These findings support the concepts of continuous usage as Bev beyond progression [110]. The reason for the discrepancy in the status of immunosuppressive cells and molecules at the recurrent stage among relevant studies remains unclear. The reason may be the difference between peripheral blood and tumors, the difference in the target of inhibition, or the response rate of targeted therapies. Tregs, PD-L1, and TAMs are upregulated in patients showing partial response [101]. Tada et al. suggested that analyses of TILs and immune cells using tumor specimens are more important than analyses of peripheral blood for investigation of cancer immunology [36]. Further investigation using tumor samples as well as peripheral blood may be required for monitoring immunosuppressive cells and molecules and seeking for predictable and prognostic biomarkers.

Future direction

VEGF plays a key role in the development of the immunosuppressive TME by inhibition of DC maturation and enhancement of immunosuppressive cells and molecules [21, 23]. Immunologically “cold” tumors are unresponsive to immunotherapies including immune checkpoint inhibitors. Anti-angiogenic therapy may change the TME into an immunological favorable “hot” microenvironment. Bev suppresses immunosuppressive cells including TAMs, Tregs, and MDSCs, and improves the migratory capacity of CTLs [14]. Theoretically, Bev could enhance the effect of PD-1/PD-L1 inhibitors (Fig. 2). Therefore, many basic research studies have demonstrated that combination therapy of VEGF/VEGFR inhibitors and PD-1/PD-L1 inhibitors induces a synergistic effect on several types of tumors including GBs, melanoma, lung cancer, and hepatocellular carcinoma [92, 96, 111]. Patients with metastatic renal cell carcinoma show improvement in antigen-specific T cell migration after combination usage of anti-VEGF and anti-PD-L1 antibodies [111]. Currently, clinical trials testing these combination therapies are being conducted for several types of tumors such as recurrent GBs, renal cell carcinoma, colorectal cancer, and ovarian cancer (NCT03024437, NCT02659384, NCT02873962, NCT02017717) [16]. This treatment strategy may lead to promising results for those malignant refractory tumors. However, high dose and long-term usage of anti-VEGF/VEGFR therapy is associated with hypoxia which is one mechanism of resistance to this combination therapy [108]. Further investigations are warranted to determine the adequate dosage and duration of anti-angiogenic agents for the combination usage with immune checkpoint inhibitors. Because immune checkpoint inhibitors and anti-VEGF/VEGFR treatment are immunological “break off” strategies, other immunotherapies such as DCs-based immunotherapy [112], tumor vaccine therapy [113], and chimeric antigen receptor T-cell therapy [114] should be added to them as “acceleration on” strategies to improve therapeutic efficacy. Furthermore, hypoxia-targeted therapy is another treatment strategy to overcome the mechanisms of resistance to immunotherapy, via suppressing Tregs, TAMs, and MDSCs [115].

As described above, reprogramming the TME to an immune-supportive microenvironment improves cancer immunotherapy [36, 49, 62–103]. To conquer therapeutic refractoriness to immunotherapy, the cross talk of immunosuppressive cells and molecules in the TME must be comprehensively regulated.

Fig. 2 Synergistic treatment strategy of VEGF/VEGFR-targeted therapy and immune checkpoint inhibitors. Cytotoxic T cells (CTLs) are excluded by components of the tumor microenvironment (TME). Immune checkpoint molecules, and immunosuppressive cells including tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs) were induced into the TME via VEGF-A. Anti-angiogenic therapy, such as bevacizumab (Bev), changes the TME into an immunological favorable microenvironment. Bev improves the migratory capacity of CTLs. Although alteration of TME after Bev administration has been still inconsistent, Bev has the possibility to suppress some immunosuppressive molecules and cells. Therefore, Bev can enhance the effect of immune checkpoint inhibitors, leading to the synergistic effect. Furthermore, immunological “acceleration on” therapies, such as dendritic cells (DCs)-based immunotherapy, tumor vaccine therapy, and chimeric antigen receptor T-cell therapy should be added to “break off” therapy including immune checkpoint inhibitors and anti-angiogenic therapy, to improve therapeutic efficacy. Bev bevacizumab, CAR chimeric antigen receptor, CTL cytotoxic T cell, DC dendritic cell, MDSC myeloid-derived suppressor cells, VEGF-A vascular endothelial growth factor, PD-L1 programmed cell death ligand 1, PD-1 programmed cell death receptor 1, Treg regulatory T cell, TAM tumor-associated macrophage

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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