Recruitment of bone marrow derived cells during anti-angiogenic therapy in GBM
Boer, Jennifer C.; Walenkamp, Annemiek M. E.; den Dunnen, Wilfred F. A.

Published in:
Critical Reviews in Oncology/Hematology

DOI:
10.1016/j.critrevonc.2014.05.001

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2014

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 04-11-2023
Recruitment of bone marrow derived cells during anti-angiogenic therapy in GBM: The potential of combination strategies

Jennifer C. Boer\textsuperscript{a}, Annemiek M.E. Walenkamp\textsuperscript{a,1}, Wilfred F.A. den Dunnen\textsuperscript{b,*,1}

\textsuperscript{a} Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
\textsuperscript{b} Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Received 9 December 2013; revised in form 10 April 2014; accepted 2 May 2014

Contents

1. Introduction ........................................................................................................... 39
2. Neovascularization in GBM .................................................................................... 39
3. The role of bone marrow derived cells in angiogenesis/vasculogenesis .............. 39
   3.1. Are BMDC an actual source of PPCs and EPCs? ............................................. 40
   3.2. The activity of monocytes as vascular modulators ........................................ 42
4. Bevacizumab treatment in GBM . ......................................................................... 42
   4.1. Bevacizumab treatment in recurrent GBM .................................................... 42
   4.2. Radiological response assessment following anti-angiogenic treatment .... 42
   4.3. Bevacizumab treatment in newly diagnosed GBM ......................................... 43
5. Angiogenic resistance following bevacizumab treatment ....................................... 43
6. Future directions: What to combine with anti-angiogenic drugs ......................... 44
   6.1. Ang/Tie-2 or CXCR4 inhibition to block TEM and TAM recruitment? ........... 44
   6.2. Blocking EPC and PPC with kinase ligand inhibitors ................................... 44
7. Conclusions . .......................................................................................................... 45
   Conflict of interest statement .............................................................................. 45
   Financial support ................................................................................................. 45
   Reviewer ............................................................................................................... 45
   References . .......................................................................................................... 45
   Biography . ........................................................................................................... 48

Abstract

Glioblastoma (GBM) is a highly vascular tumor characterized by rapid and invasive tumor growth, followed by oxygen depletion, hypoxia and neovascularization, which generate a network of disorganized, tortuous and permeable vessels. Recruitment of bone marrow derived cells (BMDC) is crucial for vasculogenesis. These cells may act as vascular progenitors by integrating into the newly formed blood vessels or as vascular modulators by releasing pro-angiogenic factors. In patients with recurrent GBM, anti-vascular endothelial growth factor (VEGF) therapy has been evaluated in combination with chemotherapy, yielding improvements in progression-free survival (PFS). However, benefits are temporary as vascular tumors acquire angiogenic pathways independently of VEGF. Specifically, acute hypoxia following prolonged

\footnote{Corresponding author. Tel.: +31 50 3610175; fax: +31 50 3619107.}
\footnote{E-mail address: w.f.a.den.dunnen@umcg.nl (W.F.A.d. Dunnen).}
\footnote{\textsuperscript{*} These authors contributed equally.}

http://dx.doi.org/10.1016/j.critrevonc.2014.05.001

1040-8428/© 2014 Elsevier Ireland Ltd. All rights reserved.
VEGF depletion induces the recruitment of certain myeloid cell subpopulations, which highly contribute to treatment refractoriness. Here we review the molecular mechanisms of neovascularization in relation to bevacizumab therapy with special emphasis on the recruitment of BMDCs and possible combination therapies for GBM patients. © 2014 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Bone marrow derived cells; Angiogenesis; VEGF; GBM; Resistance

1. Introduction

Grade 4 astrocytoma, glioblastoma (GBM), is the most frequently occurring primary malignant brain tumor in adults. Despite multimodality treatment consisting of gross total surgical resection, followed by concurrent radiochemotherapy and subsequent chemotherapy, the median survival time remains approximately 12–14 months [1,2]. GBMs are refractory to most cancer cytotoxic agents and responses are often short-lived with rapid development of resistance (Table 1).

Currently, one of the treatment options for recurrent GBM is anti-angiogenic therapy [3,4]. Given (1) the highly vascularized nature of GBM, (2) the pivotal role of vascular endothelial growth factor (VEGF) in tumor progression and (3) the slight although encouraging results of phase II clinical trials, the use of bevacizumab (Avastin, Roche, monoclonal antibody against VEGF-A) as treatment for recurrent GBM patients was consented by the Food and Drug Administration (FDA) [5]. However, as tumors may adapt to anti-angiogenic inhibitors by acquiring angiogenic pathways independent of VEGF, bevacizumab therapy produces only temporary benefits [6,7]. These pathways may include vessel normalization leading to co-option of normal vasculature, upregulation of angiogenic factors, tumor invasion and the recruitment of bone marrow derived cells (BMDC), all leading to a more aggressive tumor phenotype [6,8–10].

In this review we will specifically discuss recruitment of BMDCs and possible therapeutic strategies to circumvent this.

2. Neovascularization in GBM

Neovascularization is a phenomenon in which the formation of new blood vessels may occur through two mechanisms: angiogenesis and vasculogenesis. While angiogenesis is a process in which the propagation of new blood vessels occurs through the migration and proliferation of preexisting endothelial cells undergoing differentiation, vasculogenesis occurs through de novo formation of blood vessels. Given the highly vascularized environment in which GBMs reside, during the initial stage of tumor growth, tumor cells gain access to oxygen supply by growing along existing vessels in a process called co-option [11]. Also, tumor vessels may undergo expansion after the insertion of interstitial tissue columns into the lumen of pre-existing vessels. Subsequently opposing capillary walls protrude into the vessel lumen and the counter sites establish contact, the endothelial bilayer becomes perforated and increases its circumference (intussusception) [12,13].

GBM stem cells may also contribute to the formation of tumor vasculature by differentiating into endothelial cells [14]. In addition, tumor cells with highly invasive capacities may mimic vessels by forming fluid-conducting channels (vasculogenic mimicry) [15].

As tumor growth progresses, tumor cells migrate along blood vessels, compromising the vessel integrity by compressing and reducing the perfusion [16]. This causes hypoxia in the surrounding tissue and induces tumor cells to secrete pro-angiogenic growth factors like hypoxia inducible factor 1α (HIF1α), Placental growth factor (PIGF), VEGF and chemokine ligand 12 (CXCL12) [8,17,18].

Thus, tumor responses to oxygen depletion include several modulatory mechanisms like angiogenesis including co-option, intussusception or the more recently discovered vascular mimicry and GBM cell trans-differentiation processes. Moreover the secretion of a number of chemokines and growth factors ultimately supports the aberrant blood vessel formation. Finally, the recruitment of BMDCs occurs, which will be discussed in more detail below.

3. The role of bone marrow derived cells in angiogenesis/vasculogenesis

Evidence for the role of pro-angiogenic BMDCs in tumor vascularization was revealed around a decade ago by using angiogenic defective, tumor resistant Id-mutant mice [19]. When id-mutant mice were transplanted with wild-type b-galactosidase positive BM or vascular endothelial growth factor (VEGF)-mobilized BMDCs, tumor angiogenesis and growth was restored [19]. Moreover the pivotal role of hypoxia induced HIF1α in the recruitment of BMDCs has been elucidated by Du et al. [8]. BM cells from b-actin-enhanced green fluorescent protein (GFP) mice were transplanted into lethally irradiated Rag1-deficient mice. Subsequently HIF1α proficient (wild type (WT)-GBM) or deficient (HIF knock out (KO)-GBM) transformed mouse astrocytes were intracranially implanted. Indeed mouse brains with implanted proficient HIF1α WT-GBM cells showed up to 20% GFP+ cells while only one third of this number was found in animals with HIF KO-GBM cells [8]. These results were in line with a GBM neovascularization
study conducted by Aghi et al. who showed that hypoxia induced CXCL12 secretion is sufficient for engraftment of BMD vascular progenitors into tumor blood vessels [20].

Overall, decrease in oxygen levels is perceived by endothelial cells (Fig. 1A) that react by producing angiopoietin-2 (Ang-2), Tie-2, CXCL12, platelet derived growth factor-B (PDGF-B), bFGF and VEGF (Fig. 1B) [21–25]. Further evidence suggests that proliferation and migration of EPCs is PDGF-B dependent through PDGFRβ signaling pathway and VEGF release [26] and that PDGF-B produced by tumor cells, upregulates the production of CXCL12 from endothelial cells. In turn initial studies in pancreatic tumors reported that PDGFRβ+ PPCs recruited from the bone marrow to the tumor may produce VEGF [27]. Finally preclinical studies involving acute myeloid leukemia and GBM showed that CD45+ myeloid cells are attracted by CXCL12 (Fig. 1C) [8,28]. Therefore it may be concluded that these factors play an important role in the recruitment of BMD pericyte progenitor cells (PPCs), endothelial progenitor cells (EPCs) and myeloid CD45+ cells of monocytic lineage.

3.1. Are BMDC an actual source of PPCs and EPCs?

Currently the contribution of PCC to tumor vasculature in the context of GBM is still a matter of debate since the population of PPCs may dramatically vary depending on the tumor type and stage [29]. The presence of PPCs in the tumor vasculature has been reported in subcutaneous melanoma and pancreatic tumorigenesis models [27,30]. Specifically in the subcutaneous melanoma mouse model, BMD GFP+ cells were engrafted in recipients. In these animals the recruitment of PPC into the tumor was reported by observing BMD GFP+ periendothelial cells immunoreactive for hematopoietic markers CD11b and CD45 [30]. In GBMs a lower presence of pericytes compared to normal brain tissue is observed [31]. This might be due to decreased tight junctions and may be the reason why only modest rates of PPCs with high variability were reported throughout literature. Nevertheless signaling of the PDGF family has been proposed as key role player in the development and progression of GBMs. Hamdan et al. reported that CXCL12 induces upregulation of PDGF-B which in turn stimulates the differentiation PDGFRβ(β)+ PPCs into pericytes [32].

EPCs on the other hand have been extensively studied for their role as vascular progenitors although the true source of these cells and their actual contribution to tumor vasculature is still an ongoing debate.

EPCs were originally derived from angioblast precursors isolated from leukocyte fractions of peripheral blood [33] thus establishing for the first time that circulating human blood cells are capable of differentiating into endothelial cells. Subsequently numerous studies engaged in the effort to identify the actual origins of EPCs which resulted in an extremely difficult task as markers used to identify these putative EPCs overlap with other cell types, leaving the question on whether these cells are actual EPCs or not. This might be the reason why some authors have demonstrated major (while others minor) contribution of EPCs to the tumor vasculature [34–37]. Therefore new methodologies of EPC identification were advocated and are now based on specific cell functions, including high proliferation and colony formation capacities and not only on membrane markers [38–40]. Their proper nomenclature and isolation methodologies have been extensively reviewed elsewhere [39–42].
Fig. 1. Schematic representation of different components engaged in bone marrow derived cell recruitment. (A) Lowered oxygen levels foster necrotic areas with (B) subsequent up-regulation of numerous factors including VEGF, Ang-2, PDGF, CXCL12 and bFGF which signal to the bone marrow. (C) The bone marrow in turn releases monocytes, EPC and PPC attracted by the tumor. (D) At the tumor site EPCs and PPCs are incorporated into the tumor vasculature while monocytes turn into TEMs and TAMs in response to environmental cues. These cells in turn release a number of MMPs, growth factors and interleukins involved amongst others in ECM degradation. Abbreviations: VEGF, vascular endothelial growth factor; Ang-2, angiopoietin-2; PDGF, platelet derived growth factor; CXCL12, chemokine (C-X-C motif) ligand 12; bFGF, basic fibroblast growth factor; CXCR4, C-X-C chemokine receptor type 4; VEGFR1, vascular endothelial growth factor receptor 1; PPC, pericyte progenitor cell; EPC, endothelial progenitor cell, TEM, tie-2 expressing monocyte; TAM, tumor associated macrophage; MMP, matrix metallo protease; Up-A, urokinase plasminogen activator; IL, interleukin; ECM, extracellular matrix.

Thus, cells containing (1) highly proliferative spindle like shape, (2) colony forming capacities, (3) the expression of common endothelial cell markers such as CD34+, CD133+ VEGFR2+ and (4) CD45− [43] or CD45dim [40,44] are referred to as endothelial colony forming cells (ECFCs) (alternatively called endothelial outgrowth cells) and are currently considered as the closest cells resembling EPCs [40,45]. However the question whether these cells originate from the bone marrow, or not remains elusive. For instance Tura et al. suggested that BMD mononuclear cells formed the typical highly proliferative ECFCs with spindle like structures, yet these cells did not contain endothelial cell like markers but mesenchymal markers instead [46]. Alternatively Jiga et al. demonstrated that BMD rat ECFCs were able to re-perform revascularization in ischemic tissues. Additionally the authors found that these cells were expressing CXCR4 thus confirming their pro-angiogenic capacity [47].

To date very few studies have specifically investigated the existence of a BMD ECFC contribution to tumor vasculature in the context of GBM. Bieback et al. demonstrated (by using intravital microscopy on tumor grafts grown on skin-fold chamber in nude mice) that ECFCs were recruited to the tumor site and had the ability to incorporate into the tumor vessels [48]. However the ECFCs were grown from human cord blood and not from the bone marrow. In another study authors suggested that Hoechst labeled EPCs extracted from rat BM and implanted in the spinal column of nude mice could differentiate into endothelial and non-endothelial like cells. However a number of CD34 KDR CD31 positive cells found in the tumors were not Hoechst labeled and the exact identification of these cells was not investigated [49]. Finally results from Guo et al. who showed for the first time that EPC and hematopoietic stem cells (HSC) could be isolated from GBM. EPCs were identified based on public gene expression data profiling and marker identification was associated with specific endothelial cell functions. However the actual source of these cells was not investigated [50]. Taken together these studies suggest that in the context of GBM it is not yet known whether EPCs are actually originating in the bone marrow or not.
3.2. The activity of monocytes as vascular modulators

Previous studies showed that myeloid CD45+ cells of monocytic lineage make up the majority of BMDC and their presence is sufficient for GBM neovascularization [8]. Monocytes lodge in the perivascular area and release angiogenic factors such as angiopoietins and VEGF; possibly followed by the recruitment of BMD endothelial and pericyte progenitors, which are thought to ultimately integrate into the vasculature [27].

Mature monocytes are released in the bloodstream and differentiate into macrophages when entering the tumor [51]. Polarized macrophages include M1 and M2 macrophages, where M1 macrophages are involved in anti-tumor immunity and M2 play a prominent (i.e. supportive) angiogenic role in human gliomas [52,53]. M2 and immature monocytes may exert their function as vascular modulators by producing a number of cytokines and growth factors while not taking part in the tumor vasculature itself [6,54].

Vascular modulatory M2 macrophages, alternatively dubbed tumor associated macrophages (TAMs), produce large amounts of pro-angiogenic factors including VEGF, VEGF-C, IL-8, bFGF and proteases like urokinase plasminogen activator (uPA), matrix metallo protease (MMP)-1, MMP-2 and MMP-9 (Fig. 1D) [51,55,56]. Moreover, mutual interaction of many M2 secreted factors ultimately concurs to the formation of new blood vessels. Specifically, interaction between uPA and MMPs are involved in remodeling and breakdown of the extracellular matrix (ECM) possibly supporting vasculogenesis as matrix disruption might facilitate BMDC infiltration [54]. In addition PDGF-BB up-regulates the expression of CXCL12 [57], which in turn may lead to the accumulation of factors like MMP-9 [58].

All together these studies suggest that angiogenesis is the result of a complex network of events in which lowered oxygen levels trigger the release of a number of cytokines, MMPs and growth factors involved in the recruitment of vascular progenitor cells. In addition, vasculogenesis is triggered by vascular progenitor cells, which integrate into the blood vessels, stimulate the production of pro-angiogenic factors and elicit the recruitment of vascular modulatory cells.

4. Bevacizumab treatment in GBM

Bevacizumab has been extensively investigated in several different settings, ranging from single agent treatment to combined modality approaches in both recurrent and newly diagnosed GBMs.

4.1. Bevacizumab treatment in recurrent GBM

Several studies investigating the result of treatment with bevacizumab in recurrent glioma patients showed promising outcomes. Initially a phase II study conducted by Vredenburgh et al. found acceptable toxicity of bevacizumab. In this study, a total of 32 grade III–IV astrocytoma patients were tested, of whom 23 had GBM. The combination therapy of bevacizumab with irinotecan revealed 30% 6 month PFS with median PFS of 20 weeks in the GBM group [59]. In a second phase II trial, two small patient cohorts were investigated by Vredenburgh et al., who tested bevacizumab in combination with irinotecan in different doses and schedules. Of all the 35 patients, 57% showed at least partial response, 46% had 6 months PFS and 77% 6 months overall survival (OS) [4].

These results were further corroborated in 2 other phase II studies. The first study conducted by the National Cancer Institute (NCI) included 48 patients in which bevacizumab was investigated, resulting in 29% 6 months PFS with median PFS of 16 weeks and 57% 6 months OS with a median OS of 31 weeks [60]. The second study, a multicenter-open label noncomparative trial conducted by Friedman et al. comprised 167 patients, selected for treatment of either bevacizumab alone or in combination with irinotecan, yielding 42.6% 6 months PFS versus 50.3% if given in combination with irinotecan [3]. The observed responses were considered exceptionally high as the usual 6-month PFS rates is less than 10% in recurrent GBM [61].

Taken together these data indicated that bevacizumab could convey relatively high anti-glioma activity, which persuaded the FDA to give conditional approval of bevacizumab for use in recurrent GBM [5]. However, the lack of appropriate controls has resulted in a rejection for use in this indication by the European Medicine Agency (EMA)/Committee for Medicinal Products for Human Use (CHMP) [62].

Other anti-angiogenic drugs, including small molecule tyrosine kinase inhibitors have also been studied in GBM patients and (reviewed in [63,64]). Overall the effectiveness of small molecule TKi has been very disappointing. For example, a phase I/II study of sorafenib in combination with temsirolimus for recurrent glioblastoma or gliosarcomas showed minimal activity [65]. In a randomized controlled phase III trial the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in 325 patients with recurrent glioblastoma was investigated. This study did not meet its primary endpoint of PFS prolongation with either of these combinations [66].

4.2. Radiological response assessment following anti-angiogenic treatment

Assessing radiological response rate after anti-angiogenic treatment can be challenging. Anti-angiogenic agents can produce high radiographic response rates as defined by a rapid decrease in contrast enhancement on MRI, that is partly a result of reduced vascular permeability to contrast agents rather than a true antitumor effect, defined as pseudoresponse. Additionally in some of these patients the reduction in contrast enhancement is accompanied by
a progressive increase of nonenhancing T2 weighted/fluid attenuated inversion recovery image sequences (FLAIR), suggestive of infiltrative tumor. Therefore in 2010 response assessment criteria of high grade gliomas were updated by the Response Assessment in Neuro-Oncology Working Group (RANO). The RANO criteria assessment considers enlarging areas of nonenhancing tumor as evidence of tumor progression [67].

4.3. Bevacizumab treatment in newly diagnosed GBM

Despite variable outcomes for recurrent GBM patients, the investigation of bevacizumab therapy has progressed in the context of newly diagnosed GBM. Initial phase II single-arm studies of bevacizumab treatment, in newly diagnosed GBM patients, yielded heterogeneous results in PFS and OS. Lai et al. initially tested the toxicity and possible treatment efficacy in a cohort of 70 patients. Results showed 88% 6 month PFS in comparison to the 58% 6 month PFS obtained in the University of California, Los Angeles/Kaiser Permanente Los Angeles (KPLA) control cohort, consisting of 110 newly diagnosed patients treated with standard therapy who received bevacizumab at recurrence [68]. However no OS benefit was observed by Lai et al. in contrast to the University of California, Los Angeles/KPLA results. Further promising results came from a cohort of 51 patients tested by Narayana et al., who obtained with the addition of bevacizumab to standard therapy 85.1% 6 month PFS and an 51% 12 month OS [69]. However the outcome of phase III clinical trials,-AAglio (NCT00943826) and RTOG0825 (NCT00884741), presented at ASCO 2013 [70–73] have both indicated that bevacizumab in the setting of newly diagnosed GBM when combined with standard therapy had disappointing outcome results. In the Avaglio study, 921 patients were treated with standard therapy combined with bevacizumab or placebo. Results revealed median OS of 16.7 months in the placebo group and 16.8 months in the bevacizumab group.

Similarly in the RTOG0825 trial a total of 637 patients were randomized and the same treatment combinations were applied as for the Avaglio trial. The median OS was 15.7 in the bevacizumab group and 16.1 in the placebo group.

In conclusion these results indicate that bevacizumab treatment in addition to standard therapy and in the context of newly diagnosed GBM does not exhibit improved OS. Therefore it should not be used as first line treatment.

5. Angiogenic resistance following bevacizumab treatment

As the 2 above-mentioned randomized placebo controlled trials (Avaglio and RTOG0825) had disappointing outcomes, further studies on resistance mechanisms of bevacizumab are warranted.

It is now clear that tumor refractoriness to anti-angiogenic therapy may be described as a result of an inherent or evasive tumor resistance [6,74]. Inherent tumor resistance is defined by the presence of an intrinsic and pre-existing tumor refractoriness in which subjects exhibit no response to therapy.

In the evasive resistance, tumors may adapt to angiogenic blockade. The molecular mechanisms and cellular events underlying tumor refractoriness to therapy are still not completely understood. However emerging preclinical data demonstrate that during anti-VEGF treatment, reduced circulation of VEGF is followed by transient vessel normalization. Vessel normalization has been reported as a brief phase in which the balance between pro- and anti-angiogenic signaling is temporarily restored [75–77]. This initial phase is rapidly followed by vessel regression induced by sustained anti-angiogenic treatment [78]. While subsequent vessel regression causes rapid HIF1a up-regulation in response to acute hypoxia, which is one of the driving forces of BMDC recruitment and specifically myeloid cell infiltration in various tumors [8,22].

Several studies have been performed in the attempt to investigate the molecular mechanisms underlying tumor refractoriness after bevacizumab treatment [6,10,79,80]. In the context of BMDCs, the finding of CD11b(+)/GR(+) granulocyte infiltration appears to be the most consistent throughout literature. A recent orthotopic mouse model study by Piao et al. showed that survival of animals treated with bevacizumab in combination with sunitinib was increased as compared to bevacizumab only treatment, while combined treatment delayed macrophage infiltration until tumor progression, at which point CD11b(+)/GR(+) granulocyte infiltration was observed in both cases [79].

These results were in line with previous findings in which refractoriness of lymphoma and lung cancer cell lines to anti-VEGF treatment in subcutaneous mouse models was associated with the recruitment of tumor-associated CD11b+/Gr(+) granulocyte myeloid cells [10]. Furthermore Shojai reported that granulocyte colony-stimulating factor (G-CSF) was highly upregulated in refractory tumors of subcutaneously implanted mice. By treating animals with anti-G-CSF, they showed that the circulation of tumor-associated myeloid cells was significantly reduced, indicating the important role of G-CSF in tumor refractoriness post bevacizumab treatment.

Regardless of the cancer type, to date, the vast majority of preclinical studies indicate that bevacizumab alone does not induce prolonged tumor regression but does generate vessel normalization, which might still create a window of treatment opportunity for other agents [77].

Accumulating evidence suggests that acute hypoxia following prolonged VEGF depletion contributes to the recruitment of certain subpopulations of myeloid cells, which play a prominent role in refractoriness to anti-VEGF treatment. Therefore bevacizumab therapy might be more effective in combination with other treatment modalities as compared to single agent use.
6. Future directions: What to combine with anti-angiogenic drugs

The aim of this review was to delineate tumor refractoriness upon bevacizumab treatment from a perspective of BMDC recruitment and possibly identify combination therapies, which might result in better outcome. Therefore combination of anti-VEGF therapy with other anti-angiogenic drugs should contain compounds targeting the BMDC recruitment. Here we discuss the theoretical feasibility of anti-VEGF treatment in combination with targeted agents directed against Ang/Tie-2 axis, CXCR4, PIGF or PDGF-B.

6.1. Ang/Tie-2 or CXCR4 inhibition to block TEM and TAM recruitment?

The recruitment of TEMs (Tie2 Expressing Monocytes) is crucial to the formation of new blood vessels [81]. Previous studies have demonstrated that hypoxic conditions induce CXCL12 [8] and upregulate CXCR4 expression on TEMs (and Tumor Associated Macrophages; TAMs) [82]. Likewise, the expression of CXCL12 has also been identified as a key element for the recruitment of many other CXCR4 positive BMDCs [8] including TAMs [82]. Therefore it might be reasonable to speculate that the combination of these events ultimately leads to the recruitment of TAMs and that combining bevacizumab treatment with the blockade of TEM recruitment to the tumor site might be an option.

In addition, TAMs are currently considered key players in tumor progression and metastasis and are indicators of poor prognosis [53,56]. A number of preclinical studies have suggested that the use of the CXCR4 inhibitor AMD3100 might have positive effects on the inhibition of BMDC recruitment [8,83]. CXCR4 inhibition might provide additional benefits by directly blocking EPCs [20] and indirectly blocking PPCs, as blocking the CXCL12/CXCR4 axis hampers the PDGF-BB induced pericyte recruitment [57]. In addition the effectiveness of AMD3100 in combination with bevacizumab is currently being tested in a phase I study for recurrent GBM (NCT01339039).

Furthermore the Ang/Tie-2 pathway has also been under investigation for possible treatment options. Targeting elements of the Ang/Tie-2 pathway separately or together could be beneficial for several reasons. First, TEMs are typically characterized by the expression of Tie-2 [81,84] and the upregulation of Ang2 destabilizes vessels [85,86] thereby fostering TEM recruitment [87,88]. Second, since vessel survival following Tie-2 activation is independent of VEGF [89], dual inhibition of VEGF and Tie-2 signaling may improve clinical benefits [90]. Third, in some cases, dual inhibition of Ang1/Ang2 results in improved treatment outcome in preclinical mouse models compared to solely inhibiting Ang2 [91,92]. However, as throughout literature a growing body of evidence suggests that the balance of the vascular stabilizer Ang1 and vascular destabilizer Ang2 is critical for the nature of the emerging vascular phenotype [93] the question whether targeting of the Ang/Tie-2 pathway is beneficial or not remains unclear. The reason behind this conundrum resides firstly in the context dependent role of Ang1 expression. While some studies have found that Ang1 promotes tumor angiogenesis [94,95] others have shown that Ang1 limits vessel growth [92,96]. Secondly, promoting Tie-2 signaling through Ang2 blockade might reduce angiogenic sprouting but also stabilize tumor vasculature thereby conveying resistance to anti-angiogenic therapy if not properly coordinated. Phase I clinical trials testing inhibitors for Ang1/Ang2 together (NCT01290263, NCT01137552) or Ang2 alone (NCT01248949) are currently ongoing and might provide further clarification on this matter.

In conclusion these data suggest that currently the possibilities of bevacizumab in combination with Ang2/Tie-2 inhibitors might be less adequate compared to CXCR4 inhibitors. The latter might provide enduring therapeutic results by targeting a key element consistently involved in resistance mechanisms following GBM therapy.

6.2. Blocking EPC and PPC with kinase ligand inhibitors

Regardless of their origins, the contribution of putative EPCs and PPCs to tumor development has been described [8,97–99] as well as using monoclonal antibodies against growth factors to block the recruitment of EPCs and PPCs [100]. Specifically these cells express tyrosine kinase receptors (TKR) attracted to the production of their ligands by the tumor. Markedly the upregulation of PIGF has been reported as a result of anti-VEGF(R) therapy [101,102]. Therefore the combination of anti-PIGF with anti-VEGF therapy might be considered.

Promising results came initially from studies of Fischer et al. and were later corroborated by Van de Veire et al., both groups using the alphaPIGF monoclonal antibody (5D11D4) [18,103]. By investigating syngeneic mouse models implanted with melanoma, pancreatic or colon carcinoma cells Fischer et al. showed that growth and metastasis of these tumors was inhibited, while anti-tumor effects were amplified when combined with chemotherapy. In addition alphaPIGF monotherapy reduced macrophage infiltration up to 74% and did not cause severe hypoxia, thus preventing the angiogenic rescue system.

Markedly the results of Fischer and Veire were discordant with those from Bais et al. [104] who used similar preclinical models although not identical. With a lymphoma, melanoma, colon carcinoma and a number of other cell lines, all subcutaneously implanted and treated with the anti-PIGF antibodies C9.V2 and 7A10, Bais et al. suggested that blocking PIGF does not result in a significant reduction of tumor angiogenesis during growth of mouse primary tumors.

Contradictory results of these studies might have originated from divergent preclinical settings and the use of different antibodies not evenly specific for PIGF.
Alternatively PDGF-B has also been identified as a key element involved in mobilization of PPCs while produced by EPC [27,105]. Defects in PDGFR signaling have been consistently observed in the context of GBM [26,106–108] and anti-VEGF therapy is accompanied by upregulation of this receptor. Furthermore the PDGFRβ ligand PDGF-BB is known to induce CXCL12 upregulation [57] which may result in MMP9 production [58], associated with a higher invasive phenotype [8]. Also preclinical studies from Guo et al. showed that overexpressed PDGF-B and VEGF in an orthotopic mouse model was associated with increased capillary-associated pericytes thus showing that PDGF-B is involved in the enhancement of glioma angiogenesis [26]. The importance of PDGF-B inhibition was further shown by Jo et al., who assayed the combination of VEGF and PDGF-B in ocular angiogenic disease showing that inhibition of both VEGF and PDGF-B was more effective than solely blocking VEGF [109].

All together these results suggest that combination of anti-VEGF therapy with anti-PIGF or PDGFR–B inhibition might provide enduring results.

7. Conclusions

In this review we have explored the function of BMDCs in glioma and their activity during anti-angiogenic therapy. Essentially, anti-angiogenic therapy in GBM is known to induce hypoxia, which in turn elicits several modulatory mechanisms resulting in angiogenesis. Angiogenic events are mainly supported by the secretion of a number of chemokines and growth factors actively involved in the aberrant blood vessel formation. In turn a fundamental hallmark of vasculogenesis is BMDC recruitment. BMDC recruitment arises from a complex network of events in which lowered oxygen levels trigger the release of a number of cytokines, MMPs and growth factors involved in the recruitment of vascular progenitor cells. The latter are thought to integrate into the blood vessels, stimulate the production of pro-angiogenic factors and elicit the recruitment of vascular modulatory cells. Bevacizumab has been tested in several different clinical trials both in patients with recurrent and newly diagnosed GBM. While recurrent patients seem to partially benefit from bevacizumab treatment this is not the case for patients with newly diagnosed GBM. Preclinical studies suggest that acute hypoxia following prolonged VEGF depletion concurs to the recruitment of certain subpopulations of myeloid cells. Therefore we have discussed a number of targetable BMDC subsets, which throughout literature have consistently shown adverse activity after anti-VEGF therapy, some even in different preclinical settings. Particularly, based on the partial phenotypic overlap of receptor and ligand expression on the BMD responsive cells, combination therapies against ligands and/or their receptors might improve future treatment results in glioma patients.

Conflict of interest statement

The authors have no financial or personal conflict of interests to disclose. There are no sources of funding for this manuscript.

Financial support

Annemiek M.E. Walenkamp and Wilfred F.A. den Dunnen received a grant from the Dutch Cancer Society (grant number RUG 2010-4622)—there was no involvement from the sponsor in the decision to submit this article for publication.

Reviewer

Professor Pieter Wesseling, Department of Pathology, 1007 MB Amsterdam, Netherlands.

References


null


Biography

Wilfred F.A. Den Dunnen is currently associate professor in Neuropathology, Principal investigator at the Research School Behavioral and Cognitive Neurosciences and Coordinator of the International Research Master Clinical & Molecular Neurosciences at the University Medical Center of Groningen. He obtained his degree in Medicine at the University of Groningen in 1996. During his studies he started a Ph.D. thesis on biodegradable nerve guides for the reconstruction of peripheral nerves, which was completed in the same year as his medical degree. His main research lines concern neuro-oncology and neurodegenerative diseases. Concerning the former the focus lies on angiogenesis and proteomics. Most of his research line is conducted in collaboration with national and international partners.