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# Role of Apelin in Glioblastoma Vascularization and Invasion after Anti-VEGF Therapy: What Is the Impact on the Immune System?

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## Abstract

The limited efficacy of current antiangiogenic therapies calls for a better understanding of the specific resistance mechanisms in glioblastoma (GBM) and the urgent development of new therapeutic strategies targeting these path-ways. In this issue of *Cancer Research,* Mastrella and colleagues reported that expression of the proangiogenic peptide apelin (APLN) was decreased and GBM cell invasion was increased after anti-VEGF therapy in preclinical models of GBM. Using the mutant form of the natural apelin-13 peptide, the authors showed reduction of both angiogenesis and invasion in the GBM models, and further increased the efficacy of anti-VEGF therapy. VEGF blockade is still widely used as salvage therapy for recurrent GBM, therefore these intriguing results have potential translational implications as they point to a potential new strategy to overcome VEGF blockade resistance; however, they also raise important questions for the clinical translation of this strategy, and its impact on antitumor responses, in particular immune responses.

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor in adults. The prognosis for patients suffering from this cancer remains dismal, with a median survival of approximately 15 months after standard treatment with maximal safe surgical resection and chemoradiation. GBMs are tumors characterized by a high degree of genetic heterogeneity, cellular proliferation, invasion of brain structures, cooption of existing blood vessels, formation of abnormal vessels from existing vessels (i.e., angiogenesis), and abnormal tumor microenvironment. In addition, GBM cells usually have a low mutational burden and thus often have low neoantigen levels. This reduced tumor immunogenicity, coupled with abnormal tumor vessels and microenvironment, severely limit the ability of immune cells, especially effector T cells, to recognize tumor cells and initiate effective antitumor responses. The abnormal GBM vessels cause heterogeneous blood perfusion and hypoxia, while their high permeability causes elevated fluid pressure and vasogenic edema. This leads to preferential accumulation of immunosuppressive immune cells, for example, M2-type macrophages, microglia, and T regulatory cells (Tregs), in the GBM microenvironment. Finally, the $CD8^+$ T cells that infiltrate GBM tissues often express high

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levels of inhibitory coreceptors such as CTL antigen 4 (CTLA4) and programmed cell death 1 (PD-1) immune checkpoints. As a result of all these factors, effector T cells become dysfunctional, and are unable to mount an effective antitumor response.

The progress in our understanding of the relationship between vascular abnormalities and immunosuppression in GBM has led to multiple clinical trials of agents designed to target tumor angiogenesis (such as anti-VEGF agents), or immune checkpoints (such as anti-CTLA-4 or anti-PD-1 antibodies), or both. Unfortunately, the phase III trials conducted so far have not resulted in increased overall survival of patients with GBM receiving these experimental treatments compared with standard of care. While resistance mechanisms are multifactorial, an important factor is the abnormal GBM vasculature that limits the delivery of both drugs and T cells to the tumor parenchyma (1). The limited efficacy of current molecularly targeted therapies calls for a better understanding of the specific resistance mechanisms in the microenvironment of GBM and the urgent development of new therapeutic strategies targeted them.

Targeting VEGF pathway had initially shown promise in GBM and has been extensively tested, including in several randomized phase III trials (1, 2). These trials were also motivated by the efficacy seen with anti-VEGF drugs in colorectal, lung, gastric, liver, ovarian, or renal cancers. Unfortunately, despite benefits such as reduction in vasogenic edema associated with this disease or radiation-induced necrosis (3), anti-VEGF drugs have not resulted in prolonged survival. Moreover, many antiangiogenesis agents targeting other proangiogenic pathways have failed to show activity in GBM despite intense clinical testing over the last two decades. Immunotherapy using blockade of the PD-1 immune checkpoint, which has profoundly impacted the treatment and survival of patients with other cancers (skin, lung or head-and-neck tumors), has also failed to increase overall survival in patients with GBM. The reasons for GBM resistance to anti-VEGF and anti-PD-1 treatments that are effective for certain other cancers are not completely understood. As a result, efficacious approaches to overcome resistance to anti-VEGF and/or anti-PD-1 treatment are still being awaited.

In this issue of *Cancer Research*, Mastrella and colleagues reported that the proangiogenic peptide apelin (APLN), an endogenous ligand of the G-protein-coupled cell-surface receptor APLN receptor (APLNR), was selectively expressed in GBM compared with normal brain tissue (4). Moreover, they showed that anti-VEGF therapy resulted in decreased APLN expression and increased GBM cell invasion in preclinical models of GBM. Elegant genetic models of APLN inhibition further demonstrated the proangiogenic role of this peptide. Finally, using a mutant form of the natural apelin-13 peptide (apelin-F13A, a partial agonist with avidity for APLNR), the authors showed reduction of both angiogenesis and invasion in the GBM models that further increased the efficacy of anti-VEGF therapy. These intriguing results have potential translational implications as they point to a potential new strategy to overcome resistance to VEGF blockade in GBM, which is still widely used as salvage therapy for patients with recurrent GBM in the United States. At a more fundamental level, they also raise important questions for the clinical translation of this strategy and its impact on antitumor immune responses.

First, as discussed above, GBM vasculature is structurally and functionally abnormal, which not only causes characteristic pseudopalisading necrosis, but also leads to vasogenic edema due to focal vessel hyperpermeability (5). Vasogenic edema is usually treated with corticosteroids, which have limited efficacy and profound immunosuppressive effects. Blocking VEGF, originally discovered as a permeability factor, can alleviate, at least transiently, the vasogenic edema by normalizing the GBM vasculature. Indeed, the use of anti-VEGF therapy was associated with steroid-sparing effects in patients with GBM. Unfortunately, these benefits alone did not translate into increased survival in patients with GBM. Since Mastrella and colleagues focused their studies on structural changes in the GBM vasculature, the question is: how will targeting ALPN, alone or with anti-VEGF therapy, impact vascular function and vasogenic edema? Of note, increased expression of APLN in tumor vessels has been shown to fortify tumor vessels and induce vascular maturation in extracranial tumors (6).

Second, GBM is a highly invasive brain tumor, which makes complete removal by surgery and radiation extremely difficult, and local relapse frequently leads to disease progression and lethality. It has been reported that anti-VEGF therapy does not mitigate GBM invasion or worse, can increase invasion in some cases. Thus, targeting APLN to reduce GBM invasion, as described by Mastrella and colleagues, is an attractive strategy. However, the efficacy of targeting GBM invasion remains to be determined as so far the results of clinical studies of agents inhibiting invasion-related pathways such as MET, CXCR4, and Wnt7 have been underwhelming even when combined with anti-VEGF therapy (7–9).

Third, a most timely question is how targeting APLN/APLNR will impact immunosuppression and immune responses in GBM, as APLN has known pleiotropic effects on the immune microenvironment. Tregs promote APLN expression in endothelial cells (10). In turn, APLN induction upregulates VCAM-1 on endothelial cells of the normalized vessels, which is a prerequisite for infiltration and function of antitumor T cells. When APLN induction was combined with an anticancer vaccine that boosts the immune system and generates natural killerT (NKT) cells, the normalized vessels allowed trafficking of NKT cells to the tumor and induced a strong antitumor response (6). On the other hand, mutations leading to APLNR loss of function identified APLNR as an essential gene for success of the anti-PD-1 or anti-CTLA-4 immune checkpoint blockade therapy (11). Restoring APLNR function via JAK1/STAT1 signaling may augment IFN$\gamma$-mediated antitumor responses including T-cell trafficking across blood vessels (6, 11). This is particularly important because recent studies have not shown promising initial responses after treatment with immune checkpoint blockade (anti-PD-1 anti-bodies), particularly in the neoadjuvant (presurgical) setting in patients with GBM (12, 13). However, the results from recent correlative studies also support the notion that T-cell trafficking maybe impaired in patients with GBM (13). Even in cases where anti-PD-1 treatment altered the microenvironment of GBM and enhanced activity of $CD8^+$ T cells, the number of tumor-infiltrating $CD8^+$ T cells was insufficient to mount an effective antitumor response (13, 14).

Answering these questions and addressing these challenges will have a critical impact on the future development of more efficacious molecularly targeted therapies for GBM with or without immunotherapy. Despite the hurdles summarized above and elaborated elsewhere

(1), novel immunotherapies are being developed, including engineered cytotoxic T lymphocytes (chimeric antigen receptor T cells) targeted against a specific tumor-associated antigen, vaccines, and immune checkpoint blockers (12–16). A major concern with immunotherapy approaches is the potential aggravation of brain edema and spontaneous bleeding (13, 17), and unfortunately, the use of standard steroids to reduce edema and inflammation has been shown to compromise immunotherapy efficacy (12). Thus, future studies are required to establish new approaches to reprogram the immunosuppressive GBM microenvironment and reduce edema without compromising the antitumor immune responses. Achieving this reprogramming of GBM microenvironment will be difficult, but the emergence of targets such as APLN brings promise and new research avenues.

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