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Perspective

Meningeal lymphatic vasculature, a general target for glioblastoma therapy?

Changping Zhou[1], Han Xu[1], Jincai Luo[*]

Laboratory of Vascular Biology, Institute of Molecular Medicine, College of Future Technology, Beijing Key Laboratory of Cardiometabolic Molecular Medicine, Peking University, Beijing 100871, China

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ABSTRACT

Glioblastoma (GBM) causes nearly universal mortality as a result of the failure of conventional therapies including surgical resection, targeted radiation therapy, and chemotherapy. An increasingly important treatment option is combining immunotherapy with other therapies in both preclinical and clinical studies. The central nervous system (CNS) has been historically considered an immune privileged area, but increasing evidence, including the recent rediscovery of meningeal lymphatic vessels (MLVs), has overturned this notion. MLVs are populated by multiple immune cells and connect the CNS to the periphery by draining cerebrospinal fluid with soluble CNS antigens and immune cells into cervical lymph nodes. In the past few years, more and more studies have indicated that MLVs are involved in the regulation of inflammation and the immune response in the pathogenesis of various CNS diseases including GBM. Here, we explore the critical interlinkages between MLVs and GBM therapies including chemotherapy, radiotherapy and immunotherapy, and propose the meningeal lymphatic vasculature as a general target for GBM therapy.

In China, brain tumors account for about 2.68% (109,800) of all new cancer cases (4,064,000), while the mortality of brain tumors reaches up to 53.67% [1]. Glioblastoma (GBM) is the most common type of primary brain tumor in adults, causing nearly universal mortality and a poor prognosis (the 1-year relative survival rate is 41.4%, while the 5-year survival is only 5.8%) [2]. Given the failure of conventional therapies including surgical resection, targeted radiation therapy, and high-dose chemotherapy, finding effective therapies for GBM is therefore an urgent unmet need, although it is very challenging because of complex tumor microenvironment (TME). Notably, the TME of GBM is immune-suppressive, as shown in a very recent study that provides a comprehensive immune cellular landscape and its changes during GBM progression by single-cell RNA sequencing [3]. Consequently, immunotherapy has shown limited efficacy in GBM treatment. For example, a preclinical study of programmed cell death 1 (PD-1) blockade failed to show an obvious clinical benefit following surgery [4]. Another randomized clinical trial also reported that nivolumab (a PD-1 inhibitor) monotherapy did not improve overall survival compared with bevacizumab-treated control patients [5]. Although the results were disheartening, immune activation was detected in these data, which suggested that GBM is not completely unresponsive. Therefore, a combinatorial strategy with immunotherapy has been the consensus in clinical trials.

For ages, the central nervous system (CNS) had been commonly considered as an immunologically privileged area due to the presence of the blood brain barrier without classic lymphatic vessels. The rediscovery of meningeal lymphatic vessels (MLVs) in the dura mater has provided a new aspect of immunity in brain and CNS diseases including GBM [6–8]. While MLVs possess classic lymphatic functions, including drainage and immunoregulation, they, like a bridge, connect the brain to the cervical lymph nodes (CLNs) and are open to the trafficking of immune cells. A very recent study found a conserved three-dimensional anatomy of MLVs in mice and humans, by which MLVs exit the skull through the jugular vein foramen and drain cerebrospinal fluid into the deep CLNs [7]. In the past years, increasing numbers of studies have indicated that the meningeal lymphatic vasculature, consisting of MLVs and their draining lymph nodes, are involved in the regulation of inflammation and immune responses in pathological conditions of the CNS, such as stroke and multiple sclerosis [8]. Mechanistically, MLVs drain soluble antigens and regulate immune cell trafficking.

The above findings suggest that the meningeal lymphatic vasculature may also modulate immune responses in the progression and therapies of brain tumors. Indeed, accumulating evidence has established a close relationship between GBM and its draining lymphatics and lymph node system. Recently, several studies have begun to investigate the essentiality of MLVs in immune surveillance in brain tumors and have reported

* Corresponding author.
E-mail address: jincailuo@pku.edu.cn (J. Luo).
[1] These authors contributed equally to this work.

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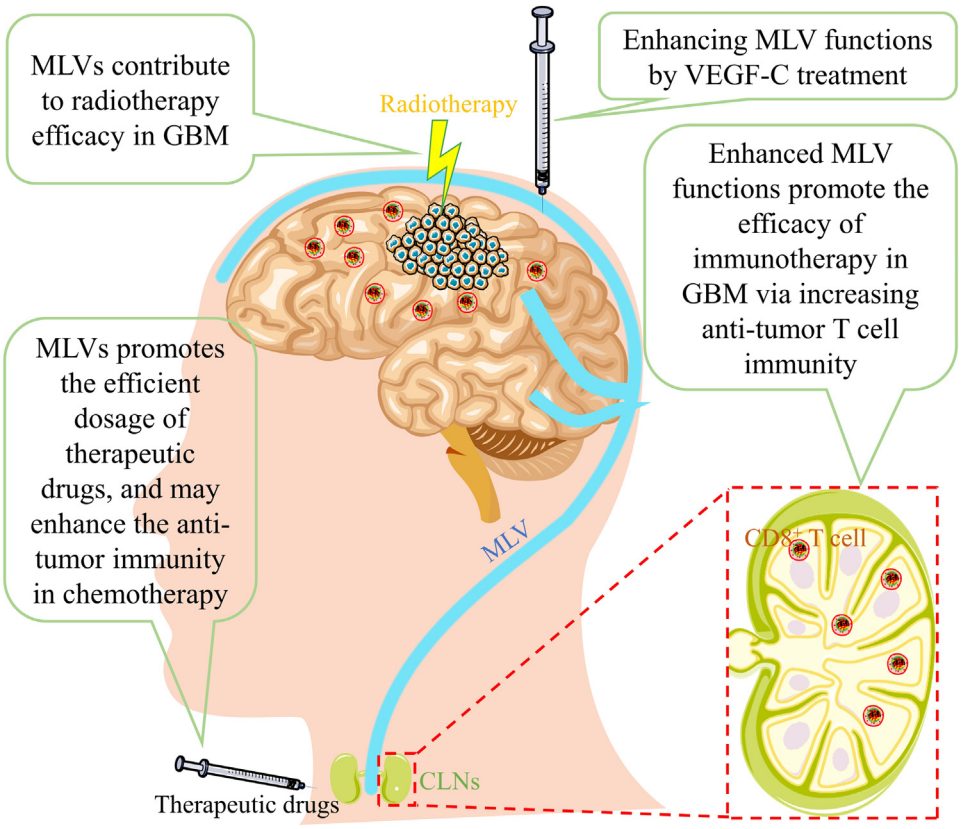

Fig. 1. The roles of MLVs in GBM therapies. Immune checkpoint therapy can provoke the immune system to exert its function in GBM. MLVs are now known to promote dendritic cell trafficking from the CNS to deep CLNs where they present antigens and activate T cells. Radiotherapy boosts the immune response in GBM in addition to directly killing tumor cells. Radiation releases more tumor antigens, which are phagocytized and drained into deep CLNs. Besides, chemotherapy also modulates anti-tumor immunity. Drug delivery has always been a challenge in chemotherapy, especially in brain tumors, while MLV transport may help to promote efficient dosage. Thus, enhancing MLV functions by VEGF-C treatment can be a general target for GBM therapy.

that vascular endothelial growth factor C (VEGF-C) combined with immunotherapy have better therapeutic efficacy in GBM in mice [9,10]. The data demonstrated that VEGF-C enhances the effect of cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or PD-1 blockade [9]. Similar supporting results were reported in our study, which showed that VEGF-C promotes dorsal meningeal lymphangiogenesis and enhances the draining function of MLVs [10]. Meanwhile, the authors found that overexpression of VEGF-C improves the immune efficacy of PD1/CTLA4 therapy. These studies show the tremendous potential of VEGF-C delivery in immunotherapy. In addition to immune checkpoint inhibition, several immunotherapy studies are ongoing, like those on a tumor vaccine [11] and CAR-T cell therapy [12]. These strategies directly target antigen-presenting cells or are closely associated with the antigen presentation process. MLVs have been shown to mediate dendritic cell trafficking, which is expected to be a potential target for combination immunotherapy [9,10].

In conventional therapies for GBM, it might be beneficial to enhance the efficacy by modulating MLV-triggered immune responses. Over the past decades, the usual techniques have been the stereotactic implantation of radioactive source beads or intraoperative radiotherapy, which increase the dose received by the glioma bed. However, radiotherapy alone failed to dramatically prolong GBM survival. Recently, we found that MLVs contribute to radiotherapy efficacy in glioma, and enhance the MLV functions induced by VEGF-C promoting the radiotherapy-modulated anti-tumor immunity [13]. Furthermore, VEGF-C mRNA significantly enhances the radiotherapy efficacy and anti-tumor immunity in glioma. Therefore, MLVs and methods that enhance their functions, such as VEGF-C, are a potential target for the improvement of the efficacy of GBM radiotherapy. For chemotherapy, multiple methods, including convection-enhanced delivery, implanted and injected modalities, and intra-arterial delivery, have been used to increase the efficient dosage of therapeutic drugs [14]. On the other hand, chemotherapy is usually combined with radiotherapy or immunotherapy in order to improve the efficacy. Studies have shown that temozolomide (a classic chemotherapy drug) affects immune functions in brain tumors, by which it induces the rapid expansion of antigen-specific T cells [15]. While MLVs also modulate similar immune responses, it is feasible to enhance chemotherapeutic efficacy by promoting MLV functions. Thus, further studies need to clarify the roles of MLVs in GBM therapy, and determine whether enhanced MLV functions contribute to the efficacy of combination therapy.

MLVs are populated by multiple immune cells [16,17], therefore increasing emphasis has been given to the MLVs-mediated dynamic alteration of cellular immune function. Expanded meningeal lymphatic vasculature induced by VEGF-C treatment changes T cell phenotypes and functionality in brain tumors [9]. The increased functional T cell infiltration of tumors can overcome the immune naivety of GBM and provide a favorable anti-tumor environment. Meanwhile, B cells are constitutively present in the dural meninges. The majority of these cells are supplied by skull bone marrow through specialized vascular connections, and dural fibroblast-like cells may regulate the survival and differentiation of early B cells [18]. This skull bone–meningeal path of B cell development may provide a constant source of B cells educated by CNS antigens. In a recent study, a B cell-based vaccine has been developed to resoundingly promote the survival and functionality of $CD8^+$ T cells; the B cells also produce tumor-reactive IgG and control tumor growth [19]. Although B cells have been shown to participate in anti-tumor immunity in GBM, the concrete mechanism is still unclear. The

special B cell source in dural meninges may provide a new view, and the contact between MLVs and B cells is worth exploring. These results suggest that MLVs actively participate in T cell-associated immune responses and may be related to B cells, which partly explains why MLVs show benefit in immunotherapy. In addition to B cells, there are also a substantial number of border-associated macrophages (BAMs) within the dura mater [16]. Different from microglia, BAMs are present in non-parenchymal tissues, are involved in immune surveillance, and support the entry of peripheral immune cells into the CNS under pathological conditions like viral and parasitic infections [20]. Besides, a recent study has demonstrated that BAMs are the third main myeloid cell population of the TME [21]. However, the relations between BAMs and brain tumors or BAMs and the dura mater are still unclear. Therefore, the precise roles of MLVs in the context of tumor immunity await further investigation.

The meninges, including the leptomeninges and the dura meninges, play a key role in CNS autoimmunity. Recently, a few studies have indicated that the leptomeninges play a more active role in orchestrating immune activity during CNS inflammation. They claim that in experimental autoimmune encephalomyelitis models and human multiple sclerosis autopsy lesions, there is a clear involvement of the leptomeninges, whereas the inflammatory reaction in the dura is significantly lower. This differential involvement of the meningeal layers is due to differences in the autoantigen availability. CNS autoantigens do not reach the dura in sufficient quantities due to the limited exchange between CNS and dura [22]. Since leptomeningeal cells line CSF spaces, including the arachnoid granulations and lymphatic drainage pathways, how the interplay between the leptomeninges and MLVs regulates CNS fluid homeostasis and immunosurveillance will be a topic of interest for scientists in the field. Nevertheless, the currently more challenging problem in this field is that we still know little about the actual role of MLVs in regulating the glioma-immune system in the native micro-environment. The use of spontaneous animal tumor models and ultimately the evaluation of the relationships between MLVs and various therapies in treating patients with glioblastomas will likely provide the solution to this key issue.

In summary, MLVs may commonly contribute to the efficacies of immunotherapy, radiotherapy, and chemotherapy for GBM (Fig. 1), suggesting that the meningeal lymphatic vasculature may serve as a general target for GBM therapy.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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Changping Zhou obtained his Ph.D. degree in the research group of Prof. Lemin Zheng in the Institute of Cardiovascular of Peking University, and currently is a postdoctoral fellow under the supervision of Prof. Jincai Luo in the Institute of Molecular Medicine of College of Future Technology of Peking University. He aims to explore the roles of lymphatics in brain tumors and cardiovascular diseases.

Jincai Luo (BRID: 09336.00.68892) received his Ph.D. from Graduate School of Medicine, University of Tokyo in 1999, under the supervision of Prof. Masabumi Shibuya. He was pointed as a principal investigator of Laboratory of Vascular Biology in 2011 after working at Institute of Molecular Medicine as Co-PI (associate professor), Peking University, for 5 years. Dr. Luo's laboratory at Peking University has focused on the studies of basic issues of vascular biology, lymphatic regulation of anti-tumor immunity, and highly interested in related translational medicine.