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REVIEW PAPER

Targeting glioblastoma-derived pericytes improves chemotherapeutic outcome

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Abstract

Glioblastoma is the most common malignant brain cancer in adults, with poor prognosis. The blood–brain barrier limits the arrival of several promising anti-glioblastoma drugs, and restricts the design of efficient therapies. Recently, by using state-of-the-art technologies, including thymidine kinase targeting system in combination with glioblastoma xenograft mouse models, it was revealed that targeting glioblastoma-derived pericytes improves chemotherapy efficiency. Strikingly, ibrutinib treatment enhances chemotherapeutic effectiveness, by targeting pericytes, improving blood–brain barrier permeability, and prolonging survival. This study identifies glioblastoma-derived pericyte as a novel target in the brain tumor microenvironment during carcinogenesis. Here, we summarize and evaluate recent advances in the understanding of pericyte's role in the glioblastoma microenvironment.

Keywords Pericytes · Glioblastoma · Blood–brain barrier · Chemotherapy

Introduction

Gliomas are tumors that arise from glial cells [1]. Glioblastoma multiforme is the most aggressive type of these tumors [2], and the major brain primary tumor in adults worldwide [3]. Glioblastoma is a highly vascularized, invasive, diffuse, infiltrating, and a drug-resistant malignant cancer with the grimmest prognosis comparing to most tumors [4]. The median survival is approximately 1 year after diagnosis, despite current therapies [5]. Only less than 5% of patients with glioblastoma survive 5 years after diagnosis [6, 7]. The few accepted risk factors for glioblastoma comprise male gender, white ethnicity, increased age, high dose of ionizing radiation, and rare genetic syndromes [8]. Presently, the initial conventional therapy of patients diagnosed with glioblastoma consists of maximal surgical resection [9]. Nevertheless, the probability of recurrence is high due to the aggressiveness, and spread of these cancer cells in the brain [10]. Therefore, resection of glioblastoma primary tumors is followed by radiotherapy, and chemotherapy [1]. Regrettably, the main chemotherapeutic agent used temozolomide, alkylating drug which sensitizes glioblastoma cells to radiation, induces a small survival benefit [11]. Limited drug delivery through the blood–brain barrier is the main reason for failure of otherwise promising compounds for glioblastoma effective treatment.

The blood–brain barrier plays key roles in brain homeostasis, and consists of highly specialized endothelial cells surrounded by pericytes and glia [12–15]. It comprises a biochemical and a physical barrier for drug delivery in the adult brain [16]. Continuous tight junctions adjoin brain endothelial cells preventing diffusion in between them [17]. The presence of this physical interface between brain parenchyma and peripheral circulation was demonstrated more than a century ago, by dye injection into the blood stream that stained peripheral organs, without achieving the brain tissue [18]. Thus, in the presence of an intact blood–brain barrier, only lipophilic molecules from the bloodstream, smaller than 400 Da, can enter the brain parenchyma by transiting across endothelial cell luminal and abluminal plasma membranes [17]. Therefore, blood–brain barrier is neuroprotective in normal conditions, blocking the entrance of noxious agents to the

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brain, being advantageous [19]. In contrast, in glioblastoma patients, this barrier limits the arrival to the brain parenchyma of several oncologic drugs including hydrophilic molecules, monoclonal antibodies, and antibody-drug conjugates [20]. As a result, although the blood–brain barrier may be partially disrupted in brain tumor patients, multiple anti-glioblastoma agents have defective delivery and distribution in the brain parenchyma contributing to cancer recurrence [21]. To reach the invasive cancer cells in the brain, circumventing and counteracting the blocking effects of the blood–brain barrier, new strategies enhancing the efficacy of glioblastoma therapy are needed.

Pericytes are defined based on their anatomical localization surrounding blood vessel walls, and communicating with endothelial cells [22–24]. Pericytes contribute to vascular stabilization [25], and blood flow regulation [26, 27]. Their role in the maintenance of functional integrity of the blood–brain barrier is well established [28]. Now, in a recent article in *Cell Stem Cell*, Zhou et al. investigated whether targeting glioblastoma-derived pericytes improves chemotherapy efficiency [29]. The authors revealed that high pericyte coverage of glioblastoma blood vessels is associated with poor response to chemotherapy in human patients, indicating that reducing pericyte coverage may improve chemotherapy efficacy. As part of pericytes in the glioblastoma microenvironment are derived from cancer stem cells [30], Zhou et al. targeted those cells by using state-of-the-art techniques, including thymidine kinase targeting system in combination with glioblastoma xenograft mouse model. These experiments revealed that elimination of glioblastoma-derived pericytes alters vascular permeability in the brain tumor, facilitating the efficient delivery of small molecules [29]. Furthermore, disruption of tumor-derived pericytes improved the anti-glioblastoma activity of etoposide, an anti-cancer drug that penetrates poorly in the blood–brain barrier, retarding tumor growth, and extending animal survival [29]. Interestingly, trying to identify specific glioblastoma-derived pericytes' molecules for pharmacological targeting, the authors discovered that bone marrow tyrosine kinase on chromosome X (BMX) is highly expressed in neoplasia-derived pericytes in comparison to normal brain pericytes. Therefore, Zhou et al. treated glioblastoma-bearing mice with ibrutinib, a reported potent inhibitor of BMX. This therapy increased vascular permeability, improving the diffusion of small molecules into the tumor. Strikingly, ibrutinib treatment enhanced the effectiveness for a poor blood–brain barrier penetrating drug in a mouse model with glioblastoma xenograft, prolonging the survival of those mice [29]. Here, we discuss the findings from this study, and evaluate recent advances in our understanding of the pericyte' biology in the glioblastoma microenvironment.

Perspectives/future directions

Glioblastoma immune microenvironment

Cancer mouse models try to mimic human disease; they expand greatly our capacity to decipher mechanistic details in vivo, and play a critical role in the development of novel therapies for glioblastoma. Immunocompromised mouse models preventing host immune rejection are widely used in glioblastoma research, as in the study by Zhou et al. [29], due to their certainty of glioblastoma initiation, speed of glioblastoma development, and simple establishment. Nevertheless, multiple compounds with significant anti-cancer effects in immuno-deficient mouse models do not work in human patients [31]. This may be due to the crucial roles that the immune system plays during tumor development [32, 33]. Because of the distinct tumor microenvironment in immunocompromised mice compared with humans, it is not possible to evaluate the effect of a specific therapy on the tumoral immune system by the use of this kind of model [34]. Thus, immunodeficient mouse models should be used in combination with syngeneicly transplanted and genetically engineered immune-competent mouse models in order to minimize the disadvantages of each model. Future studies should reveal whether targeting glioblastoma pericytes in mice with active immune system also improves effectiveness of chemotherapy.

Pericytes, in addition to their role in the maintenance of functional integrity of the blood–brain barrier [28], have several immune functions [35]. They express adhesion molecules associated with the control of immune cells trafficking, such as VCAM-1 and ICAM-1 [36], and produce multiple chemokines important for immune cells functions [37–39]. Pericytes regulate lymphocytes activation [40–43], and attract innate leukocytes to exit through the sprouting blood vessels [44]. Pericytes also can affect blood coagulation, contribute to the clearance of toxic cellular byproducts, and have direct phagocytic activity as macrophages [45–52]. Importantly, recently it has been shown that in the brain pericytes are important for immunomodulation in the glioblastoma microenvironment [53, 54]. Therefore, it should be explored what is the effect of pericyte' blockade on the immune cells that reside in the glioblastoma microenvironment, and how this affects brain tumors' progression. Pericyte roles are complex, and our understanding of the cross-talk between pericytes and immune cells still remains restricted. Therefore, elucidating the details of the cross-talk between pericytes and different immune cell subsets in the brain tumor microenvironment is key to the development of anti-glioblastoma therapies.

Targeting pericytes in the glioblastoma microenvironment

Pericytes are essential for the formation of new blood vessels, angiogenesis, during tumor growth [55]. For this reason, strategies targeting pericytes have been considered as anti-angiogenic treatments for different types of tumors [56]. Nonetheless, till now, clinical cancer studies with pericyte' blockade have failed to ameliorate patients' outcome [57, 58]. Higher pericytes' coverage was related to better prognosis in some patients [59]. Importantly, in certain conditions, pericyte targeting even enhanced tumor metastatic progression [60–63]. Thus, the strategy to block pericytes requires a careful examination of glioblastoma and its microenvironment morphology and functional properties to determine whether a particular agent is having an effect. Zhou et al., using the thymidine kinase targeting system in glioblastoma xenograft mouse model, blocked exclusively glioblastoma-derived pericytes, which correspond to only part of pericytes present in the glioblastoma microenvironment [30]. Future studies should examine the effect of blocking also the other subset of pericytes non-glioblastoma-derived present in the tumor microenvironment. A better understanding of the molecular differences between glioblastoma-derived and non-glioblastoma-derived tumoral pericytes may reveal specific targets for anti-glioblastoma therapies (Fig. 1).

Glioblastoma pericyte' role as a stem cell, and as a niche cell for cancer stem cells

Pericytes are highly plastic cells [64], having the capacity to differentiate into distinct cellular populations, including osteoblasts [65], myoblasts [66], adipocytes [67], fibroblasts [68], smooth muscle cells [24], and chondrocytes [38]. Due to their multipotency, pericytes are promising targets for tissue regeneration and repair [25]. Recently, it has been shown that pericytes also have neurogenic potential, being able to generate neural and glial cells [69–73]. It remains completely unexplored pericyte' plasticity in the glioblastoma microenvironment. Future studies should reveal whether pericytes have the ability also to form other stromal cells in the brain tumor microenvironment which may influence glioblastoma progression. Also, it remains undefined whether pericytes can become malignant cells, or whether glioblastoma-derived pericytes may de-differentiate into glioblastoma cancer cells in the glioblastoma microenvironment.

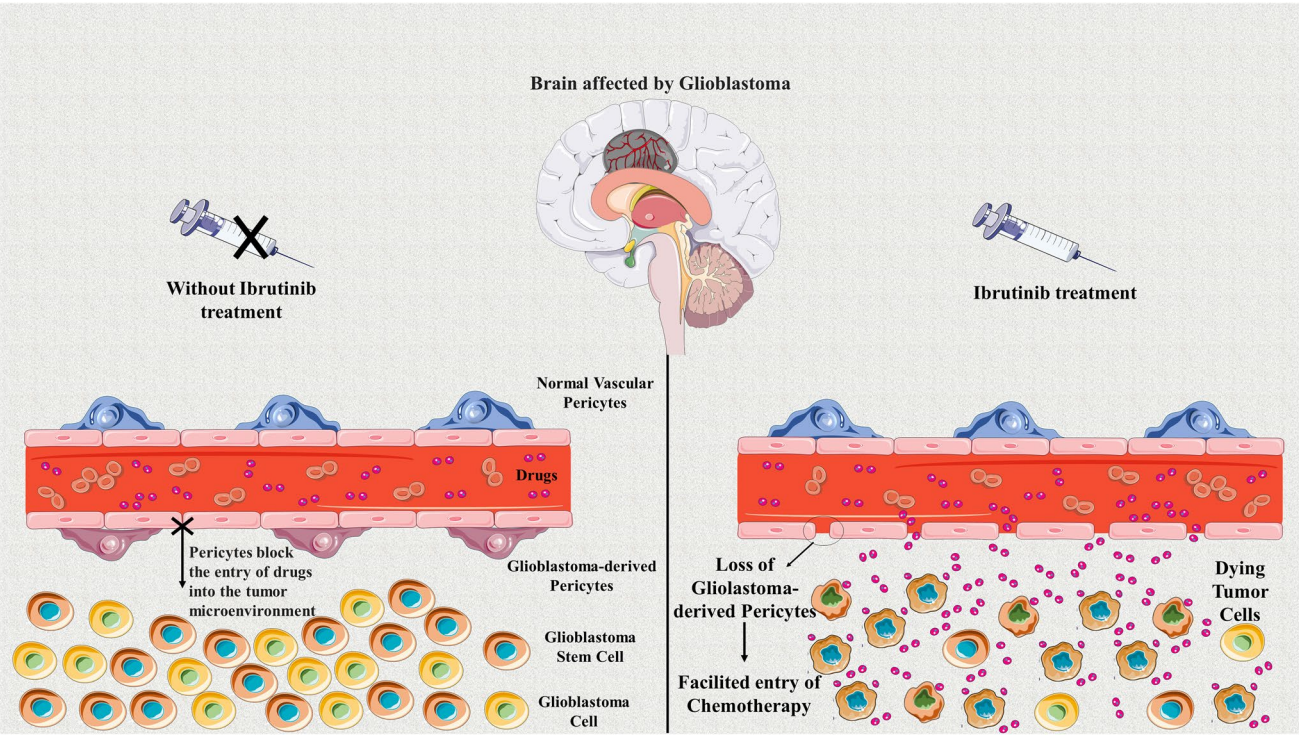

Fig. 1 Glioblastoma-derived pericytes as a novel therapeutic target. Pericytes associated to cerebral blood vessels residing within the brain tumor microenvironment can be subdivided into two big subpopulations: glioblastoma-derived and non-glioblastoma-derived. The study of Zhou et al. now reveals that targeting glioblastoma-derived pericytes improves chemotherapy efficacy [29]. Ibrutinib, a reported potent inhibitor of BMX, enhances the effectiveness of chemotherapy via reducing pericytes coverage. With the appearance of state-of-art modern technologies, future studies will reveal in detail all cellular components and their interaction with glioblastoma cells in the brain tumor microenvironment

These hypotheses can be evaluated by genetic fate-tracing pericyte-specific mouse models to access pericyte plasticity in vivo in the glioblastoma microenvironment.

In addition to its capacity to function as stem cells [74–76], pericytes can regulate the functioning of other stem cells, being important components of stem cell niches [38, 77–81]. The characteristic that several stem cells share is that they are concentrated in the proximity of blood vessels, which shelter them from noxious stimuli, and control the equilibrium between self-renewal and differentiation [38, 82]. Similarly, it has been suggested that glioblastoma stem cells as well reside in a perivascular niche that stimulate their self-renewal and long-term growth [83]. The role of pericyte as a niche cell for glioblastoma stem cells has not been explored yet. Identification of signals produced by pericytes important for glioblastoma stem cells maintenance may reveal whether, how, and when pericytes regulate glioblastoma stem cells behavior. If the pericyte functions as a cancer stem cell niche component as well, it raises interesting questions: Does targeting glioblastoma pericytes affect this role as well? Are glioblastoma stem cells attracted to the pre-existing pericytes, or do they previously generate glioblastoma-derived pericytes to support themselves? Also, it remains to be evaluated whether targeting glioblastoma-derived pericytes assists chemotherapy to eliminate glioblastoma stem cells.

Perivascular cells heterogeneity in the glioblastoma microenvironment

Even though pericytes are characterized by their anatomical perivascular localization, not all perivascular cells are pericytes [84, 85]. Several cells that may share molecular markers, including the ones used by Zhou et al. [29], with pericytes have been described as perivascular: e.g., macrophages [86–88], adventitial cells [89], smooth muscle cells [38], and fibroblasts [90]. Zhou et al. ablated genetically glioblastoma-derived pericyte-based desmin, a type-III intermediate-filament protein, expression. However, this marker could refer to other cell populations. For instance, desmin is known to be expressed in astrocytes, and other glial cells in the central nervous system [91–93]. Although none of brain pericyte markers are specific, when used in combination they clearly distinguish pericytes from other cell types [94]. Importantly, neoplastic astrocytes also may express desmin [95], therefore, it is possible that Zhou et al. eliminated malignant astrocytes, when ablation was done using the desmin-driven HSV-TK system [29]. Future studies will need to clarify whether the genetic ablation of pericytes was essential for the chemotherapeutic improvement, as probably other cell populations were affected as well.

Pericytes are heterogeneous in their morphology, distribution, molecular markers, origin, and function [96]. Pericytes associated with distinct blood vessel types differ in their morphology, markers, and function [38, 97–99]. At least two pericyte subsets have been described in the brain: type-1 and type-2 pericytes distinguished based on their Nestin-GFP expression [68, 100]. Interestingly, not all central nervous system pericytes express desmin [101]. Thus, in addition to non-glioblastoma-derived pericytes, glioblastoma-derived pericytes not-expressing desmin were not targeted by the desmin-driven HSV-TK system [29]. Whether only a fraction of pericytes is important for the maintenance of blood–brain barrier integrity remains to be studied.

Molecular targeting of glioblastoma pericytes

Recent advances in the understanding of the molecular and cellular mechanisms involved in glioblastoma progression pave the way for the development of targeted therapies that would decrease chemotherapeutic toxicity, while increasing therapeutic efficacy. Targeting pericytes have been proposed as a therapy in several cancers, due especially to their angiogenic potential. Unfortunately, experimental data do not invariably anticipate success at the clinic. Zhou et al. revealed that BMX is upregulated in glioblastoma-derived pericytes [29]. However, several cell types may express BMX in addition to pericytes [102, 103]. BMX was not conditionally deleted from glioblastoma pericytes or from other cell populations that express it in the glioblastoma microenvironment, so there is no direct evidence that pericytes will be the only/main functionally important target when blocking BMX. Transgenic mouse models have been applied to study specific cell populations within distinct tissue-microenvironments [104, 105]. The ability, not only to eliminate cells, but to delete single genes in specific cellular populations in adult mice has allowed us to answer specific questions regarding the roles of molecules derived from different cell subsets in the regulation of physiologic and pathologic processes. The exact molecular mechanisms in which pericytes are involved during glioblastoma progression in vivo are yet not completely clear, and will need to be revealed in future studies. The generation of BMX-floxed mice to be crossed with pericyte-specific inducible CreER driver will allow us to specifically delete this molecule in pericytes in vivo. In addition to studies in genetic mouse models, transcriptomic and single pericyte analysis represents fundamental tools that will help us develop targeted therapies for pericytes in the glioblastoma microenvironment during different stages of cancer progression.

Efforts are underway in the field to identify tumoral pericytes inhibitors that will influence uniquely the tumor niche. Zhou et al. proposed to use the FDA-approved drug ibrutinib to disrupt selectively glioblastoma-derived pericytes [29]. Ibrutinib, previously known as PCI-32765, is a potent inhibitor of BMX. Yet, ibrutinib also have significant

activity against 19 other kinases, including BLK, BTK, ITK, TEC, EGFR, ERBB2, and JAK3 [106]. Possibly, for this reason, ibrutinib use have reported side effects which may limit its use, such as hypertension, atrial fibrillation, bleeding, diarrhea, infection, arthralgia, and skin toxicity [107–111]. Therefore, the discovery of new molecular targets within pericytes, not expressed by other cells, will lead to the development of more effective, less toxic drugs. A deeper characterization of ibrutinib effects on the glioblastoma microenvironment should be performed. Are cancer stem cells, angiogenesis, other stromal and inflammatory cells also affected? Future studies should explore the most effective use of ibrutinib in glioblastoma patients. Need to be defined: chemotherapeutic drugs that are effective in combination with ibrutinib; the timing of its use; and the optimal dosage.

Conclusion

The study by Zhou et al. reveals glioblastoma-derived pericytes as a novel important target in the glioblastoma microenvironment. However, our understanding of pericytes biology in the brain tumor microenvironment still remains limited, and future studies should shed light on the complexity and interactions of different cellular components of the glioblastoma microenvironment during carcinogenesis. A great challenge for the future will be to translate experimental data into humans. Improving the availability of human glioblastoma samples will be essential to reach this goal.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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