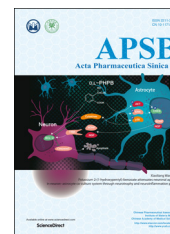




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REVIEW

# Remodeling the blood–brain barrier microenvironment by natural products for brain tumor therapy



Xiao Zhao<sup>a</sup>, Rujing Chen<sup>a</sup>, Mei Liu<sup>a</sup>, Jianfang Feng<sup>a</sup>, Jun Chen<sup>b</sup>,  
Kaili Hu<sup>a,\*</sup>

<sup>a</sup>*Murad Research Center for Modernized Chinese Medicine, Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

<sup>b</sup>*Key Laboratory of Smart Drug Delivery, Fudan University, Ministry of Education, Shanghai 201203, China*

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## KEY WORDS

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**Abstract** Brain tumor incidence shows an upward trend in recent years; brain tumors account for 5% of adult tumors, while in children, this figure has increased to 70%. Moreover, 20%–30% of malignant tumors will eventually metastasize into the brain. Both benign and malignant tumors can cause an increase in intracranial pressure and brain tissue compression, leading to central nervous system (CNS) damage which endangers the patients' lives. Despite the many approaches to treating brain tumors and the progress that has been made, only modest gains in survival time of brain tumor patients have been achieved. At present, chemotherapy is the treatment of choice for many cancers, but the special structure of the blood–brain barrier (BBB) limits most chemotherapeutic agents from passing through the BBB and penetrating into tumors in the brain. The BBB microenvironment contains numerous cell types, including endothelial cells, astrocytes, peripheral cells and microglia, and extracellular matrix (ECM). Many chemical components of natural products are reported to regulate the BBB microenvironment near brain tumors and assist in their treatment. This review focuses on the composition and function of the BBB microenvironment under both physiological and pathological conditions, and the current research progress in regulating the BBB microenvironment by natural products to promote the treatment of brain tumors.

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\*Corresponding author.

E-mail address: [kaili-hu@163.com](mailto:kaili-hu@163.com) (Kaili Hu).

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## 1. Introduction

Brain and central nervous system (CNS) tumors are the most common neoplasm among those 0–19 years old, with an average annual age-adjusted incidence rate of 5.42 per 100,000<sup>1</sup>. The annual incidence of adult glioblastoma is 7.2 per 100,000, making it the most common adult primary intrinsic brain tumor<sup>2</sup>. The BBB excludes drugs from entering the brain, due to the special structure of the microvasculature. Despite many methods of surgical resection, radiotherapy, and chemotherapy for treatment, outcomes have remained dismal<sup>3</sup>. Recently, remodeling the tumor microenvironment has become a promising way of enhancing tumor therapy for various advanced cancers; however, research on the effects of BBB microenvironment on brain tumor therapy is still in its infancy. The BBB microenvironment includes endothelial cells, astrocytes, peripheral cells, microglia, and extracellular matrix (ECM). These cells also play a key role in controlling the formation and morphology of brain tumors. Regulation of the BBB microenvironment by natural products shows increasing potential for assisting in the treatment of brain tumors. Natural products affect a number of factors secreted by some tumor-associated cells and influence tumor biology. In this review, we describe the composition and function of the BBB microenvironment and highlight the effects of natural products on regulating the brain tumor BBB microenvironment to treat brain tumors. The signaling pathways, factors, interactions described in this review provide a perspective on regulating the BBB microenvironment for brain tumor therapy.

## 2. The composition and function of the BBB microenvironment

The BBB is an important structure which maintains the balance of the CNS microenvironment and maintains the normal functioning of the brain<sup>4</sup>. The BBB microenvironment (Fig. 1) is constituted of endothelial cells, astrocytes, pericytes, macrophage, fibroblasts, neuronal cells, basal membranes, microglia, and other cell types<sup>5</sup>. In addition, there are many transporters on the BBB, including

P-glycoprotein (P-gp) and other multidrug resistance-related proteins<sup>6,7</sup>, which can reduce the apparent distribution of drugs in the brain.

Astrocytes are involved in nerve signal transmission, nutrient transport, maintaining the balance of brain microenvironment and ECM ion balance buffering. Astrocytes also participate in the inflammatory response of the CNS<sup>8,9</sup> and maintain the integrity of the BBB<sup>10</sup>.

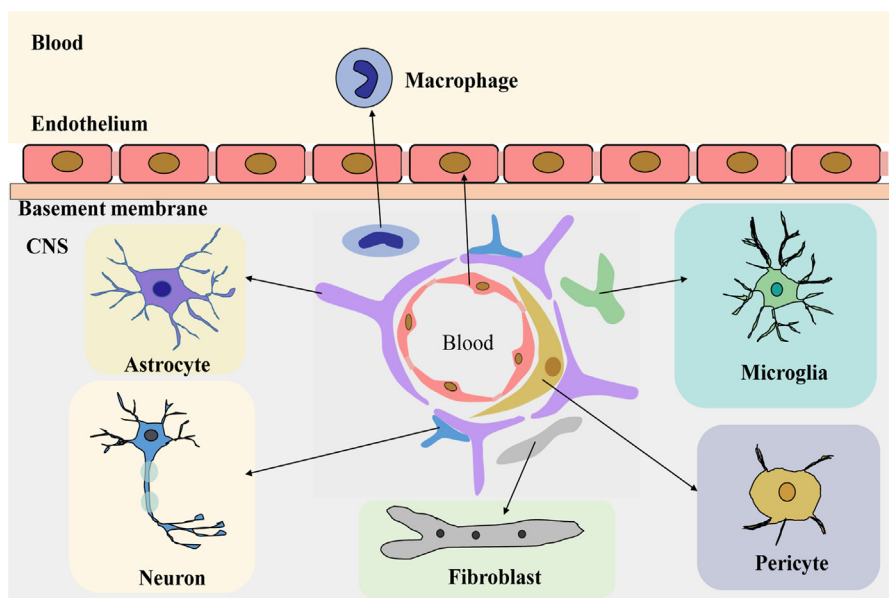
Peripheral cells are multifunctional cells, with immune function in the CNS neurovascular unit<sup>11</sup>. Peripheral cells surround the endothelial cells and play an important role in the BBB microenvironment and in maintaining the BBB function by secreting growth factors and ECM.

Microglia are a kind of long-standing immune cell in the human brain. They can stimulate the opening of BBB, leukocyte extravasation, and angiogenesis<sup>12</sup>. Fibroblasts, when co-cultured with glioblastoma cells, can induce production and activation of matrix metalloproteinase MMP2, and its activators membrane type 1 metalloprotease (MT1-MMP) and MT2-MMP<sup>13</sup>, which affect the growth progression of gliomas<sup>14</sup>.

Other cells like endothelial cells in the BBB microenvironment maintain the normal function and integrity of the BBB by forming tight junctions that limit transcytosis<sup>4</sup>. CNS neurons bind chemicals and convey electrical signals. They can regulate the ionic microenvironment of the synaptic and axonal regions of the nerve cell, which are essential to the nerve signal transduction<sup>15</sup>. The basement membrane is attached as a support tissue to the neurovascular unit cells, forming a substrate for cellular differentiation and gene expression<sup>9</sup>.

## 3. The formation, morphology and classification of brain tumors

Benign tumors are nodular, lobulated or cystic with a clear border and often with an envelope. Brain tumors are usually solitary. Under electron microscopy, the orthotopic brain gliomas have mitochondria with dense matrix cohesive structures, indicating an active state. The mitochondria extend and tightly connect to the



**Figure 1** The composition of the blood–brain barrier microenvironment.

endoplasmic reticulum<sup>16</sup>. Fibrous astrocytomas show vascular abnormalities, including fibrosis, hyaline degeneration and angiogenesis under optical microscopy. There are fenestra and vesicles among capillary endothelial cells<sup>17</sup>.

The formation of brain metastases can be divided into several consecutive processes, including epithelial-mesenchymal transition, escape from the primary tumor, entry into the vascular circulation system, survival in the blood, interaction with cerebral vascular endothelial cells through the BBB extravasation, the formation of metastatic tumor sites, and colonization in the brain<sup>18</sup>. An important condition for colonization is the formation of a metastatic niche, which is facilitated by angiogenesis stimulated by the interaction between tumor cells and endothelial cells. Tumor cells and surrounding inflammatory cells infiltrating into tumor tissue are able to produce a class of angiogenic factors, such as vascular endothelial growth factor (VEGF). These factors can stimulate endothelial cell division, proliferation, and migration, promote vascular basement membrane degradation, induction of host capillary germination and increase capillary permeability.

The most common brain tumor is glioma, which is derived from glial cells, including glioblastoma multiforme (GBM), astrocytoma, oligodendrocyte glioma and ependymoma. GBM is a kind of highly invasive primary brain tumor. Its malignant cells usually infiltrate into surrounding tissue and destroy cerebral blood flow through a variety of biomechanical and biochemical mechanisms<sup>19</sup>. It is of poor prognosis with high recurrence rate, and survival time general less than two years<sup>20,21</sup>. It is a highly aggressive tumor with a large number of neovessels, necrosis and intense resistance<sup>22</sup>. In addition, microglia and macrophages are present in polymorphic glioblastomas, promoting tumor growth<sup>23</sup>. Astrocytoma is the most common glioma, accounting for 70% to 80% of gliomas. It can grow anywhere in the brain or spinal cord.

#### 4. The glioma microenvironment

The tumor microenvironment is a dynamic network, including tumor cells, surrounding cells, and ECM and interstitial tissue, and it is a key factor affecting tumor metastasis<sup>24</sup>. The glioma microenvironment includes glioma-associated microglia and macrophages which are the most abundant immune cells in the tumor. The glioma microenvironment is a potential therapeutic target<sup>20,25</sup>. In addition, primary tumors have a strong host response at the beginning and contain a large number of immune cells, such as microglia and tumor-infiltrating macrophages. The process of tumor pathology not only is controlled by the gene composition, but also depends on the interaction with immune cells in the tumor microenvironment. It is well known that malignant tumors are immunosuppressive diseases, in which malignant tumor cells can control immune cells to support their growth and glioma-associated immune cells can promote cancer development by supporting angiogenesis and promoting tumor cell invasion, proliferation and survival<sup>26</sup>. The composition of brain tumors' BBB microenvironment is the same as the normal BBB microenvironment. While tumor blood vessels are highly irregular, the distance between the blood vessels is much greater than in the normal brain<sup>27</sup>.

##### 4.1. Cell components

###### 4.1.1. Microglia promote tumor growth in the progression of gliomas

Microglia, accounting for up to 30% of the weight of a brain tumor, are an important component of the microenvironment<sup>28</sup>.

Microglia infiltrate most of the gliomas. They release cytokines, leading to degradation of the ECM and stimulation of the signaling pathways that promote glioma cell invasion<sup>29</sup>. The complex microenvironment of malignant gliomas is dynamic, and plays an important role in promoting tumor growth in the progression of gliomas. Kees et al.<sup>30</sup> demonstrated that small glial cells isolated from tumor cells indeed supported the growth, migration, and invasion of tumor cells. The impact of microglia on glioma migration might relate to the production of MT1-MMP that is produced by microglia in response to soluble factors released from glioma cells. Microglia cells produce abundant pro-MMP-2, which can be quickly converted into matrix metalloprotease 2 (MMP-2) by MT1-MMP. Glioma cells also release MMP-2 that is promoted by MT1-MMP released from microglia<sup>31</sup>. Besides, microglia can promote the proliferation of glioma cells by increasing the activity of MMP-2 before invasion. In the glioma microenvironment, the morphology of microglia changes from a branch-like form into an amoebiform. The motility of microglia around tumor blood vessels is high, indicating an interaction between microglia and tumor blood vessels<sup>32</sup>.

###### 4.1.2. Astrocytes activate the glioma microenvironment

Astrocytes, which are the main stromal cells in the brain, can activate the glioma microenvironment, leading to the generation of a layer of activated astrocytes around the glioma. Activated astrocytes are an essential part of the glioma microenvironment<sup>33</sup>. It has been demonstrated that glioma cells activate astrocytes with upregulation of WNT/ $\beta$ -catenin signaling that can increase migration and invasion capabilities of glioma cells. In addition, glioma cells can stimulate adjacent astrocytes to degrade ECM and to promote tumor invasion<sup>34</sup>. Non-neoplastic astrocytes can be transformed in an active glioma microenvironment, and then secrete factors that affect the biological characteristics of the tumor<sup>28</sup>. Tumor cells located in the microenvironment often interact with astrocytes to promote tumor growth by secreting stromal cell-derived factor<sup>35</sup>, and astrocyte elevated gene<sup>36</sup>, both of which are overexpressed in human brain tumors<sup>37,38</sup>.

Astrocytes also play an important role in the process of tumor metastasis. By *in vitro* experiments it has been confirmed that astrocyte-derived molecules have a tumorigenic effect<sup>39</sup>. An immunohistochemical study of human glioma biopsies revealed that astrocytes were located around gliomas<sup>40</sup>. Neurological disorders are often accompanied with astrocyte hypertrophy and aggregation, and an increase in the number of astrocytes<sup>41,42</sup>. The glioma can restore the proliferation properties of astrocytes. For example, under pathological conditions activated astrocytes can proliferate<sup>43</sup> and astrocytes around the tumor, instead of removing glutamate with neurotoxicity, will respond to glioma cell signaling and release glutamate<sup>44</sup>. Gliomas can use astrocytes to secrete MMP and to enhance glioma cell invasion through soluble cascades<sup>45–47</sup>. Astrocytes can also produce and release pro-MMP-2, which can be activated by cellular MT1-MMP.

###### 4.1.3. Macrophages promote tumor growth with poor prognostics

Macrophages are also an important part of the glioma microenvironment. Tumor-associated macrophages have been proven to promote tumor growth with poor prognostics<sup>48</sup>. According to the homing characteristics of macrophages, they can pass through the endothelial barrier to the brain tumor site by secreting cytokines in the diseased tissue<sup>49</sup>. Macrophages can be a potential target for

brain tumor therapy. A study of intracranial injection of GL261 cells into Fas-induced macrophage-depleted transgenic mice showed that the mice had a lower tumor mitotic index, lower microvessel density, and slower growth of the tumor<sup>50</sup>. Macrophages are predominantly classified as type M1, classically activated macrophage, and M2, alternatively activated macrophage. M1 macrophages play a role in immune surveillance by secreting pro-inflammatory cytokines and chemokines, and full-time presentation of antigens to participate in positive immune responses. M2 type macrophages have only weak antigen presenting ability and act in the immune regulation by the secretion of inhibitory cytokines interleukin-10 and/or tumor growth factor  $\beta$  (TGF- $\beta$ ).

#### 4.1.4. Endothelial cells enhance glioblastoma stem cell proliferation and migration

Endothelial cells can secrete interleukin-8 to enhance glioblastoma stem cell proliferation and migration<sup>51</sup>. Endothelial cells can emit physical signals by directly contacting glioma cells, and communication by paracrine signals<sup>52</sup>. Normal endothelial cells maintain the integrity and normal function of the BBB. The endothelial cell is the most important structural component of the BBB, and changes in the phosphorylation state of the tight junction protein (ZO-1 or occluding) are critical to the control of BBB vascular permeability<sup>53</sup>. In areas of bulky tumors, the connection of endothelial cells is very loose and almost lacks integrity. In the invasive peripheral regions of a tumor, the close connection of endothelial cells is slightly leaky, while in the sparsely invaded regions distant from the tumor bulk, the connection of endothelial cells is very tight<sup>54</sup>.

Other cell components like peripheral cells are essential for the formation of microenvironment and survival of tumor-associated epithelial cells, promoting the growth of malignant tumors<sup>55</sup>. Brain tumors can cause detachment of peripheral cells and astrocytes, and dynamic sensory pressure of pericardial cell<sup>11</sup>. Neurons surrounding gliomas in rats have a shrunken dendritic arbor and severe functional changes such as increased spontaneous activity and decreased visual response.<sup>56</sup> As the glioma worsens, neurons are slowly depleted<sup>57</sup>.

## 4.2. ECM

ECM includes fibronectin, tumor growth factor (TGF) chemokines, laminin and hyaluronic acid, among which fibronectin, laminin and hyaluronic acid can promote the adhesion and migration of glioma cells<sup>58,59</sup>. Chemokines in glioma cells, such as chemoattractant protein families, interleukin families, EGF, TGF and tenascin, can stimulate signal transduction of malignant tumors through their receptors<sup>60</sup>. Fibronectin regulates cell adhesion, growth, metastasis, proliferation and wound healing<sup>61</sup>. Interleukin and EGF are found overexpressed in gliomas<sup>62-64</sup>.

### 4.2.1. Fibronectin

Fibronectin is an ECM protein that has important physiological and pathologic function in development and adulthood. Fibronectins subtypes, extra domain-A (EDA) and extra domain-B (EDB), are important markers of angiogenesis and play a vital role in the development of tumor cells. EDA is 100% homologous between human and mouse, while EDB is identical between human and mouse<sup>65</sup>. EDA plays an important role in a variety of processes, including cell adhesion<sup>66</sup>, myofibroblast differentiation<sup>67</sup>, cell

cycle, and mitotic signal transduction<sup>68</sup>. EDB knockout will lead to cell growth delays<sup>69</sup>. EDB can regulate the expression of VEGF, endothelial cell proliferation, and tubule formation<sup>70</sup>. According to solubility and tissue distribution, fibronectin has two main forms: one is circulating blood plasma fibronectin, and the other is cell fibronectin. Both can be aggregated into the ECM of connective tissues<sup>71</sup>.

### 4.2.2. Lactic acid

Healthy cells rely on mitochondrial oxidation of carbohydrate molecules to release ATP. The fast growth of tumor tissue leads to insufficient oxygen supply, transforming the energy source of tumor cells from an aerobic oxidation pathway to an anaerobic one. Anaerobic oxidation produces a large amount of lactic acid released into the extracellular matrix, which is responsible for the formation of an extracellular acidic microenvironment<sup>72</sup>.

### 4.2.3. Fatty acids

Tumor cells can convert nutrients to acetyl-CoA under the action of tumor genetic signals. Acetyl-CoA forms fatty acids through biochemical reactions under the action of the acetyl-CoA carboxylase and fatty acid synthetase, and fatty acids can directly or indirectly promote the proliferation and survival of tumor cells<sup>73</sup>.

### 4.2.4. Tenascin

Tenascin is an ECM protein that is ubiquitously expressed in gliomas, particularly in glioblastomas<sup>63</sup>. It participates in CNS development<sup>74</sup> and contributes to tumor cell adhesion, invasion, migration and proliferation<sup>75,76</sup>.

### 4.2.5. Vasoactive peptides

Vasoactive substances in brain tumors are more likely to be activated than in normal brain<sup>77</sup>. Vasoactive peptides can increase the penetration of the drug to the brain tumor site, and they include leukotriene and bradykinin<sup>78</sup>.

### 4.2.6. Others

Another ECM, EGF, is the earliest discovered growth factor, and plays an important role in regulating cell growth, proliferation and differentiation. It can stimulate thymus regeneration, accelerate lymphoid T cell-, B cell-, and phagocytic cell-formation, improve immune function and phagocytosis of cancer cells, and thus treat cancer and tumors. The human EGF receptor 3 is a target for anti-tumor therapy. Interleukin receptors are overexpressed in malignant gliomas. Targeting interleukin receptors can promote tumor cell death via elongation factor 2 inhibition<sup>64</sup>. TGF- $\beta$  is a protein that regulates various aspects in tumor growth and survival<sup>64</sup>. It can inhibit epithelial and endothelial cell growth, promote ECM expression, such as collagen and fibronectin, and inhibit ECM degradation.

## 4.3. The correlation between glioma and BBB in the occurrence and development of glioma

The BBB microenvironment is composed of endothelial cells, astrocytes, microglia, pericytes, as well as the ECM, and various chemokines that are secreted in the brain. In the occurrence and development of glioma, functionality changes of the BBB and supporting cells lead to BBB dysfunction. The compromised BBB allows an influx of inflammatory cytokines, including TNF- $\alpha$ , TGF- $\beta$  and interleukins, to enter the brain. Chemokines can recruit

immune cells (astrocytes, macrophages, etc.) from the blood or from within the brain to secrete MMP-2 and MMP-9 that alter BBB permeability. The tight junction protein occludin is vulnerable to attack by MMPs, which seems to have implications in glioma. In addition, in the occurrence of glioma, the brain tumor capillaries form a blood–brain tumor barrier (BBTB) that is variably distinct from the BBB and includes existing and newly formed blood vessels that contribute to the delivery of oxygen and nutrients to the tumor and facilitate glioma cell migration to other parts of the brain<sup>54</sup>. In low-grade gliomas, the normal vascularization and the function of the BBTB remains mostly intact and resembles the BBB as under normal conditions<sup>79</sup>. However, in high-grade gliomas, BBTB is characterized by major alterations of normal vascular function resulting in a disrupted, ‘leaky’ BBTB, which may allow more inflammatory cytokines to enter the brain<sup>80</sup>. The brain endothelial cell is highly reactive because it serves as both a source of, and a target for, reactive oxygen species (ROS) and inflammatory proteins. Astrocyte end-feet cover over 99% of cerebral capillaries<sup>81</sup>, leading to critical cell–cell interactions that directly regulate BBB characteristics and are fundamental for homeostasis, defense, and regeneration of the CNS<sup>82</sup>. The physical dislocation of astrocytes from the blood vessels and subsequent encasing of vessels by gliomas disrupts astrocyte–vascular coupling and will result in loss of astrocytes function. In addition, aberrant expression of chemokines, like IL-1, by glioma cells can activate astrocytes<sup>83</sup>.

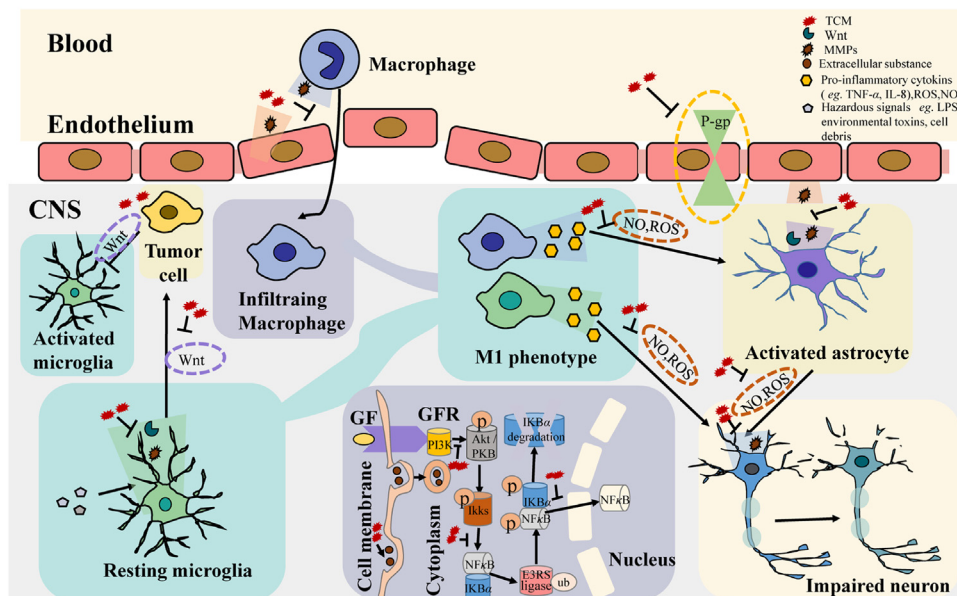
Microglia, the primary immune cells of the brain, are distributed in the CNS and are activated in response to systemic inflammation and several CNS pathophysiologies<sup>84</sup>. An activated microglia-associated tumor microenvironment will lead to glioma progression. Activated microglia are associated with not only altered tight junctions protein expression and increased BBB permeability, but also produce high levels of neurotoxic and proinflammatory mediators, such as nitric oxide (NO) and TNF- $\alpha$ , all of which result in cell injury and neuronal death<sup>85,86</sup>. Neuroinflammation

and oxidative stress lead to BBB breakdown, which are implicated in the pathogenesis of CNS disease<sup>87</sup>.

Pericyte activation mainly results from the widespread parenchymal diffusion of factors produced locally by the glioma, from glioma-derived factors such as exosomes delivered by the systemic circulation<sup>88</sup>, or instead, from the action of elevated intracranial pressure resulting from hypoxia. Svensson et al.<sup>89</sup>, showed that pericytes become activated in most areas of the brain in response to GL261 mouse gliomas. Non-tumor-derived pericytes infiltrate the glioma extensively and integrate with the vasculature. Montagne et al.<sup>83</sup> showed that pericyte injury and possibly early degeneration correlate with increased BBB permeability within the hippocampus, a region known to be affected by pericyte loss and BBB breakdown on post-mortem tissue analysis.

## 5. Natural products regulating the BBB microenvironment

Surgical treatment, chemotherapy, and radiation therapy are common treatments for brain tumors. Surgical treatment is generally used for benign tumors with high survival rates. Chemotherapy has made great progress, but due to the barrier function of BBB, brain tumor chemotherapy is still subject to many restrictions. Chemotherapeutics can enter into tumor cells only after passing the vascular endothelium and is dependent upon their lipophilicity. This process reduces the speed and efficiency of drug action. Regulation of the BBB microenvironment by natural products shows increasing prospect for assisting in the treatment of brain tumors. Natural products can regulate the BBB microenvironment by acting on P-gp, endocytosis, and MMPs, etc. (Fig. 2). In the BBB microenvironment, MMPs can be secreted by macrophages, endothelium, astrocytes, microglia, and neurons. Endocytosis is a common process for transporting extracellular material into the cells, which happens in endothelial cells<sup>90</sup>, astrocytes<sup>91</sup>, macrophage<sup>92</sup>, pericytes<sup>93</sup>, fibroblasts<sup>94</sup>, neuronal



**Figure 2** Natural products regulate the blood–brain barrier microenvironment by (1) downregulating MMPs; (2) inhibiting P-gp; (3) reducing NO and ROS production; (4) promoting endocytosis; (5) downregulating the WNT pathway; and (6) regulating the PI3K/Akt/BDNF/TRKB-ERK/NF- $\kappa$ B signaling pathway.

cells<sup>95</sup> and microglia<sup>96</sup>. P-gp is expressed on endothelial cells and functions as a transporter in the BBB. Generation of NO and ROS is a feature of genuine immune system cells as well as other cells involved in immune reactions. The WNT/ $\beta$ -catenin signaling pathway not only is involved in many biological processes of other components of BBB microenvironment but also contains oncogenes and tumor suppressor genes, which are closely related to the occurrence of tumors.

### 5.1. Modulating MMP-2 and MMP-9

MMP-2 and MMP-9 belong to a family of MMPs which are secreted by endothelial cells, astrocytes, pericytes, fibroblasts, neuronal cells, and microglia and function to dynamically balance the degradation and remodeling of ECM. In addition, MMP-9 binds to CD44 to release stored TGF- $\beta$ 1, and may be involved in angiogenesis by releasing VEGF.

#### 5.1.1. Shikonin

Shikonin, extracted from *lithospermum*, is an active derivative of anthraquinone. Zhang et al.<sup>97</sup> showed that shikonin inhibited the migration and invasion of glioma cells by inhibiting the expression of MMP-2 and MMP-9, which are secreted by microglia and can promote the proliferation and invasion of glioma<sup>98</sup>. Glial-to-mesenchymal transition proteins play a prominent role in tumor invasion, while shikonin can reduce the expression of these proteins and is a naphthoquinone with anti-tumor properties. Matias et al.<sup>99</sup> showed that a combination of shikonin and temozolomide can decrease glioblastomas-derived cells in their proliferation and migration capacities. These decreases were followed by the suppression of glial-to-mesenchymal transition through a reduction in expression of MMP-2 and MMP-9 via inactivation of PI3K/AKT signaling. Wang et al.<sup>100</sup> showed that shikonin can inhibit the over-expression of MMP-9 and effectively protect brain by regulating inflammatory responses and maintaining BBB permeability. Shikonin can inhibit the MMPs generated by both BBB microenvironment components and tumor-derived cells.

#### 5.1.2. Osthole

Osthole is an active substance extracted from the common *cnidium* fruit which is an umbelliferous plant. Osthole can inhibit proliferation and promote the apoptosis of U87 cells by regulating the expression of miR16 and inhibiting the expression of MMP-9<sup>101</sup>. Duan et al.<sup>102</sup> showed that osthole can inhibit inflammatory reactions, reduce MMP-2 activity and activate mitogen-activated protein kinase (MAPK) cascades. In addition, several studies showed that osthole can regulate MMP-2 and MMP-9 in the A549 human lung cancer cells and breast cancer cells<sup>103–105</sup>. It can also regulate MMPs secreted by myocardial cells, vascular endothelial cells, and smooth muscle cells<sup>106</sup>.

#### 5.1.3. Resveratrol

Resveratrol is a polyphenolic antioxidant found in peanuts, grapes, and red wines. Despite the low bioavailability of resveratrol, its antitumor effect has been demonstrated both in cell experiments *in vitro* and murine models<sup>107–109</sup>. The invasion and metastasis of glioblasts in early glioblastoma are highly related to glioblastoma progression and recurrence. Resveratrol can reduce glioblast invasion by inhibiting PI3K/Akt/NF- $\kappa$ B signaling and decreasing MMP-2 expression<sup>109</sup>. Hao et al.<sup>110</sup> showed that resveratrol can

inhibit proliferation and migration of human retinal pigment epithelial cells in a concentration-dependent manner with down-regulation of MMP-9. Jiao et al.<sup>111</sup> proved that resveratrol can inhibit the invasion of glioblastoma-initiating cells *via* the inhibition of PI3K/Akt/NF- $\kappa$ B signal transduction and the suppression of MMP-2 expression. Wei et al.<sup>112</sup> showed that resveratrol can attenuate the BBB dysfunction *via* regulation of MMP-9 to maintain the integrity of BBB. Resveratrol can not only regulate MMP, but also inhibit PI3K/Akt/NF- $\kappa$ B signal.

#### 5.1.4. (+)-Aeropylsinin-1

Aeropylsinin-1 is extracted from sponge *Aplisina aerophoba* and inhibits differentiation and proliferation of epithelial cells. Aeropylsinin-1 can also decrease gene expression of MMP-2, and inhibit the migration and invasion of glioma cells<sup>113</sup>. In previously published work describing aeropylsinin-1 as a potent anti-angiogenic compound, two molecular targets for its effects, namely, MMP-2 and urokinase were identified<sup>114</sup>. Martínez-Poveda et al.<sup>115</sup> confirmed MMP-2 as a molecular target of aeropylsinin-1, which can inhibit the expression of MMP-2.

#### 5.1.5. Oligomeric procyanidins

Oligomer procyanidins, natural components from grape seeds, have been shown to have an inhibitory effect on VEGF and MMP-2. It can also reduce tumor invasion through an HIF-1 $\alpha$ -dependent way<sup>116</sup>.

### 5.2. Regulating PI3K/AKT/BDNF/TRKB-ERK/NF- $\kappa$ B signaling pathway

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophic family of growth factors, plays an important role in the development, differentiation and maintenance of neurons. BDNF can promote neuronal cell survival in a concentration-dependent manner. Tropomyosin-related kinase B (TRKB) is a receptor protein involved in the development and maturation of the CNS<sup>117</sup>. BDNF has a high affinity for TRKB and was demonstrated to promote the protein expression of TRKB<sup>118</sup>. Members of the TRKB downstream signaling cascade, including PI3K/PKB and ERK/MAPK, have been reported to be responsive to BDNF<sup>119,120</sup>. Several studies have assumed that BDNF largely activates the ERK/MAPK pathway<sup>121–123</sup>. In comparison, other studies have reported that activation of the ERK/MAPK pathway leads to cell death and PI3K/PKB is the main pathway involved in the protection of neurons induced by BDNF<sup>124</sup>. Both pathways are critical for neuroprotection induced by BDNF. Nuclear factor- $\kappa$ B (NF- $\kappa$ B), a transcription factor usually activated by inflammatory stimuli and cellular stresses, has an important role in regulating the survival and growth of the cell. NF-E2-related factor-2 (NRF2), a transcription factor, mediates cytoprotective antioxidant responses and thus prevents cells from the damage induced by ROS and other harmful chemicals in various types of cancers<sup>125</sup>. The serine/threonine kinase AKT is a downstream effector of PI3K and is involved in cell survival and anti-apoptotic signaling<sup>126</sup>. Recently, a study demonstrated that cell signaling is mediated by PI3K/Akt *via* induction of the NF- $\kappa$ B, which is associated with cell migration, proliferation and angiogenesis<sup>127</sup>. NF- $\kappa$ B provides a mechanistic link between cancer and inflammation and might also regulate tumor angiogenesis and invasiveness<sup>128</sup>.

### 5.2.1. Tenuifoliside A

Tenuifoliside A is an active ingredient extracted from *Polygala tenuifolia*. Brain glioma-derived factors can improve the migration and proliferation of microglia by promoting overexpression of focal adhesion kinase and activating the PI3K/Akt pathway, thus promoting microglia accumulation in the region of the tumors. The signal from the brain glioma cells can activate the ERK/p38MAPK pathway, inhibit STAT1 and NF- $\kappa$ B signaling, and cause microglia immunodeficiency<sup>129</sup>. Dong et al.<sup>130</sup> found that tenuifoliside A can increase the phosphorylation of ERK and AKT by blocking the signaling pathway of ERK and PI3K and increase the release of BDNF. The results showed that tenuifoliside A provided a neuroprotective effect on C6 glioma cells by regulating the BDNF/TRKB-ERK/PI3K-CREB signaling pathway.

### 5.2.2. Betulinic acid

Betulinic acid is a pentacyclic triterpenoid extracted from white birch. It can directly target mitochondria and reduce the mitochondrial membrane potential<sup>131</sup>. In addition, betulinic acid can also inhibit the NF- $\kappa$ B signaling pathway to suppress inflammation and modulate the immune response in glioma<sup>132</sup>. Clinical studies demonstrated overexpression of NF- $\kappa$ B as well as downregulation of the P53 network in metastatic tumors. Shankar et al.<sup>133</sup> showed that it induces apoptosis by stabilizing P53 and downregulating NF- $\kappa$ B pathway in human prostate cancer cells. Su et al.<sup>134</sup> showed that betulinic acid can suppress the expression of c-Myc, cyclin-D1, BCL-xL and the downstream gene targets of NF- $\kappa$ B and STAT3.

### 5.2.3. Withania somnifera

*Withania somnifera* is also known as Indian ginseng. *Withania somnifera* extract can inhibit microglia invasion by decreasing the production of inflammatory cytokines such as NF- $\kappa$ B and activator protein 1, and reduces TNF- $\alpha$ /ROS production, downregulating MMPs<sup>135</sup>. Sun et al.<sup>136</sup> showed that *Withania somnifera* played a role in immunomodulatory effects. It suppressed oxidative and inflammatory responses in microglial cells by downregulating the NF- $\kappa$ B and upregulating the NRF2 pathways at the same time. Kataria et al.<sup>137</sup> proved that *Withania somnifera* reduced the intracranial tumor volumes *in vivo* and suppressed the tumor-promoting proteins NF- $\kappa$ B, p-AKT, VEGF, heat shock protein 70, polysialylated form of neural cell adhesion molecule (PSA-NCAM), and cyclin D1 in the rat model of orthotopic glioma allograft.

## 5.3. Inhibiting P-gp or pinocytosis

P-gp, an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity, is expressed on endothelial cells. It is responsible for decreasing drug accumulation in multidrug-resistant cells and often mediates the development of resistance to anticancer drugs. P-gp also functions as a transporter in the blood–brain barrier. Pinocytosis, known as fluid endocytosis and bulk-phase pinocytosis, is a mode of endocytosis in which small particles are brought into the cell, forming an invagination, and then suspended within small vesicles. Inhibition of P-gp function or facilitating pinocytosis may enhance chemotherapeutics uptake and help in glioma therapy.

### 5.3.1. Procyanidine

Procyanidine is isolated from the bark of *Pinus massoniana*. Procyanidine is extensively studied for the effective inhibition of P-gp on the BBB, which can increase the accumulation of some drugs in the brain<sup>138</sup>. It significantly increases the accumulation of rhodamine 123 in cells by inhibiting its efflux in a dose-dependent manner<sup>139</sup>. Zhao et al.<sup>140</sup> showed that procyanidine reverses P-gp-associated multi-drug resistance by inhibiting the function and expression of P-gp through downregulation of NF- $\kappa$ B activity.

### 5.3.2. Scillarenin

Scillarenin is an active ingredient extracted from the *scilla*. Scillarenin can inhibit P-gp activity in the BBB endothelial cells, improving the efficacy of tumor therapy<sup>141</sup>.

### 5.3.3. Kainic acid

Kainic acid can change the microenvironment around the blood vessels by increasing the pinocytosis of endothelial cells and opening the BBB<sup>142</sup>.

## 5.4. Inhibiting WNT/ $\beta$ -catenin

The WNT/ $\beta$ -catenin signaling pathway itself contains an oncogene and a tumor suppressor gene, which are closely related to the occurrence of tumor. Activating WNT/ $\beta$ -catenin signaling pathway will accelerate tumor growth. Lin et al.<sup>143</sup> proved that silencing of  $\beta$ -catenin in mesangial cells *in vitro* using siRNA decreased the mRNA expression levels of the pro-apoptotic proteins (cleaved caspase-3 and BAX) and the fibrosis-associated proteins (fibronectin, and collagens I/III/IV). In addition,  $\beta$ -catenin silencing attenuated TNF- $\alpha$ -induced apoptosis in mesangial cells. Many investigations suggested that WNT/ $\beta$ -catenin signaling is importantly involved in pathogenesis of fibrotic diseases<sup>144</sup>. For instance, the expression of  $\beta$ -catenin is significantly increased in keloid tissues, and the stimulation of fibroblasts by TGF- $\beta$  results in upregulation of WNT/ $\beta$ -catenin signaling<sup>145</sup>.

### 5.4.1. *Rhodiola crenulata*

*Rhodiola crenulata*, a well-known medicinal Tibetan herb, is mainly grown in high-altitude regions of Tibet, Sichuan and Yunnan in China. Recently, increasing numbers of studies have been published on the potential pharmacological activities of *Rhodiola crenulata*<sup>146</sup>. *Rhodiola crenulata* extracts can effectively suppress proliferation, stimulate differentiation, and eliminate tumor sphere formation of GBM cells *in vitro*. The mechanism may be related to the inhibition of WNT/ $\beta$ -catenin signaling pathway<sup>147</sup>. Bassa et al.<sup>148</sup> showed that *Rhodiola crenulata* can reduce the transcriptional activity of  $\beta$ -catenin and positive responses of estrogen receptor, leading to reduced proliferation and tumor sphere formation.

### 5.4.2. *Trichosanthin*

Trichosanthin is an active ingredient extracted from the roots of melon. Trichosanthin is also a plant toxin belonging to the family of ribosome-inactivating proteins. It has various biological and pharmacological activities, including anti-tumor and immunoregulatory effects<sup>149</sup>. It has dose-dependent inhibitory effect on the invasion, migration and proliferation of human glioma cells and can induce tumor cell apoptosis by the inhibition of WNT/ $\beta$ -catenin signal pathways<sup>150</sup>.

On one hand, activated WNT/ $\beta$ -catenin signaling pathways directly facilitate the invasion, migration and proliferation of human glioma cells, and induce tumor cell apoptosis. On the other hand, the pathways decrease the levels of fibrosis-associated proteins (fibronectin and collagens I/III/IV) by the inhibition of WNT/ $\beta$ -catenin signal pathways. These will facilitate the treatment of glioma.

### 5.5. Adjusting the NO level

As is known, NO is an endothelium-derived relaxing factor and contributes to vessel homeostasis by inhibiting contraction and growth of vascular smooth muscle, platelet aggregation, and leukocyte adhesion to the endothelium. In addition, NO has been recognized as one of the most versatile players in the immune system. It is involved in the pathogenesis and control of tumors, autoimmune processes and chronic degenerative diseases. The role of NO in the immune system is simply defined. NO is a product of macrophages activated by microbial compounds, cytokines, or both. It is derived from the amino acid L-arginine by the enzymatic activity of inducible nitric oxide synthase (iNOS or NOS2) and functions as a tumoricidal molecule *in vitro* and *in vivo*<sup>151</sup>. Generation of NO is a feature of genuine immune system cells (dendritic cells, NK cells, mast cells and phagocytic cells including monocytes, macrophages, microglia, Kupffer cells, eosinophils, and neutrophils) as well as other cells involved in immune reactions (such as endothelial cells, epithelial cells, vascular smooth muscle cells and fibroblasts)<sup>152</sup>.

#### 5.5.1. Salicin

Salicin is an active substance isolated from the twigs of *Salix glandulosa* Seemen. It was shown that salicin can inhibit the production of NO due to the carboxyl structure of C-7 and the acetyl structure of C-3. It can also increase the production of nerve growth factor in C6 glioma cells<sup>153,154</sup>. Kong et al.<sup>155</sup> showed that salicin can reduce ROS production and activation of the extracellular signal-regulated kinase pathway.

#### 5.5.2. Lignan glycosides

Lignan glycosides are isolated from the roots of *Wasabia japonica*. They can induce neuroprotective effects by stimulating the nerve growth factor in C6 glioma cells. It can stimulate lipopolysaccharide level in BV2 cells to affect NO levels<sup>156,157</sup>.

### 5.6. Effects on oxidative stress

Oxidative stress is a state of BBB microenvironment under interaction with glioma, and is defined by an imbalance between increased levels of ROS and a low activity of antioxidant mechanisms. An increased oxidative stress state can induce damage to the cellular structure and potentially destroy tissues. Oxidative stress can activate a variety of transcription factors, including NF- $\kappa$ B, P53, AP-1,  $\beta$ -catenin/WNT, and NRF2. The activation of these transcription factors leads to the expression of over 500 different genes, including those for growth factors, chemokines, inflammatory cytokines, cell cycle regulatory molecules, and anti-inflammatory molecules<sup>158</sup>. Recently, considerable evidence has demonstrated that ROS are involved in the relationship between cancer and chronic inflammation<sup>159,160</sup>. Indeed, an important characteristic of tumor promoters is their ability to recruit inflammatory cells and to stimulate them to generate

ROS<sup>161</sup>. Reuter et al.<sup>158</sup> clearly demonstrated the role of ROS state in different phases of tumorigenesis, such as cellular transformation, proliferation, invasion, angiogenesis, promotion, survival and metastasis.

#### 5.6.1. $\beta$ -Caryophyllene

$\beta$ -Caryophyllene can reduce neuronal damage by reducing oxidative stress injury and inflammatory cytokine-induced BBB damage<sup>162</sup>, maintaining BBB integrity. Park et al.<sup>163</sup> found that  $\beta$ -caryophyllene oxide not only inhibited the activation of the PI3K/AKT/mTOR/S6K1 signaling cascade, but also caused the activation of ERK, JNK, and P38 MAPK in tumor cells.  $\beta$ -Caryophyllene oxide induces increased ROS generation from mitochondria, which is associated with the induction of apoptosis.

#### 5.6.2. Curcumin

Curcumin is the main active ingredient isolated from food spices. Under a hypoxic state it can increase the permeability of BBB by upregulating the expression of heme oxygenase-1 (HO-1) in microvascular endothelial cells<sup>164</sup>. In addition, Xiao et al.<sup>165</sup> showed that curcumin can protect neurons from TNF- $\alpha$ -triggered excessive ROS production and cellular apoptosis. Correspondingly, it promoted mRNA expression of anti-oxidative enzymes like HO-1, catalase and superoxide dismutase-2. Santos-Parker et al.<sup>166</sup> showed that curcumin improved resistance artery endothelial function by increasing vascular NO bioavailability and reducing oxidative stress, as well as conduit artery endothelial function.

#### 5.6.3. Shizukahenriol

Shizukahenriol is a natural product isolated from the *Chloranthus henryi* Hemsl. It activates NRF2, which inhibits oxidative stress. In addition, it can induce the expression of NRF2-related antioxidant HO-1, glutamate-cysteine ligase, which consists of both the modifier and catalytic subunits in BV-2 microglial cells. This natural product can also inhibit the production of inflammatory molecules, such as NO and TNF- $\alpha$ , and inhibit translocation of NF- $\kappa$ B and P65 to the nucleus<sup>167</sup>.

## 6. Outlook

Despite the progress that has been made by chemotherapy, we are still far from desired therapeutic effects due to the special structure of the BBB. Regulation of BBB microenvironment will play an important role in assisting the treatment of brain tumors. Many studies have used natural products to regulate the BBB microenvironment, most of which focused on regulating oxidative stress, MMPs, WNT/ $\beta$ -catenin and PI3K/AKT/BDNF/TRKB-ERK/NF- $\kappa$ B pathways (Table 1). In fact, there are many other factors that can be affected by natural products in the BBB microenvironment. Further encapsulation of natural products into nanoparticles has been proven to be effective for *in vivo* studies<sup>168</sup>. Microglia and macrophages in the tumor microenvironment are potential therapeutic targets. Natural products can be used to target microglia and macrophages to remodel the tumor microenvironment for better treatment of brain tumors. However, the mechanisms of BBB regulation through natural products has not been fully explored. Further study on the mechanism by which these natural substances regulate BBB permeability are still needed.



**Table 1** Relationships between BBB constituents and different cytokines during the occurrence and development of glioma.

BBB element	Function	Related cytokine
Endothelial cells	Maintaining the BBB function	MMP
Astrocytes	The inflammatory response of the CNS <sup>8,9</sup> and maintaining the integrity of the BBB <sup>10</sup>	MMP, TGF- $\beta$ , AEG-143, IL-1 $\beta$ , IL-17, TNF, CXCL12 <sup>94–96</sup>
Pericytes	Secreting growth factors and ECM and absorbing soluble molecules into the cerebrospinal fluid <sup>9</sup>	MMP, platelet-derived growth factor- $\beta$ , (PDGF $\beta$ ), IL-1 $\beta$ , IL-6, TNF- $\alpha$ , ROS, NO <sup>97–102</sup>
Microglia	Stimulating the opening of BBB, leukocyte extravasation and angiogenesis <sup>12</sup>	MMP, TNF- $\alpha$ , IL-1 $\beta$ , CCL2, CXCL1, CXCL10 <sup>103</sup>
Macrophages	Promoting tumor growth with poor prognostics <sup>52</sup>	MMP, IL-10, TGF- $\beta$ , NO, TNF- $\alpha$ <sup>104,105</sup>
Fibroblasts	Inducing production and activation of MMP <sup>13</sup>	MMP, IL-6, IL-8 <sup>106,107</sup>
Neurons	Increasing spontaneous activity and decreasing visual response <sup>60</sup>	MMP

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