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Blood-Brain Barrier Interfaces and Brain Tumors

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In the developing brain, capillaries are differentiated and matured into the blood-brain barrier (BBB), which is composed of cerebral endothelial cells, astrocyte end-feet, and pericytes. Since the BBB regulates the homeostasis of central nervous system (CNS), the maintenance of the BBB is important for CNS function. The disruption of the BBB may result in many brain disorders including brain tumors. However, the molecular mechanism of BBB formation and maintenance is poorly understood. Here, we summarize recent advances in the role of oxygen tension and growth factors on BBB development and maintenance, and in BBB dysfunction related with brain tumors.

Key words: Blood-brain barrier, Brain angiogenesis, Barriergenesis, Tight junctions, Oxygen tension, Glioblastoma

MOLECULAR COMPOSITION OF THE BBB

The vascular system in the brain is characterized by the BBB of endothelial cells, which is a protective barrier with low permeability (Risau and Wolburg, 1990; Wolburg and Lippoldt, 2002). The endothelial barrier is supported by tight junctions, which prevent a free exchange of soluble factors between endothelial cells and brain microenvironments, and to maintain brain homeostasis.

Tight junctions in the BBB are composed of transmembrane proteins including occludin, claudin and junctional adhesion molecules (JAMs), and cytoplasmic proteins linked to the actin-based cytoskeleton (Gloor *et al.*, 2001) (Fig. 1). The transmembrane proteins mediate cell adhesion and are thought to constitute the intramembrane and paracellular diffusion barriers.

Occludin is a ~65 kDa integral membrane protein, whose presence at the BBB is correlated with increased electrical resistance across the barrier and decreased paracellular permeability (Furuse *et al.*, 1993). Occludin is highly and continuously distributed at cell-cell contacts in brain endothelial cells (Hirase *et al.*, 1997). In occludin-deficient mice, tight junctions are not altered morphologically. Therefore, it seems that mature cells need occludin to regulate rather than to establish their barrier properties (Saitou *et al.*, 2000).

Claudins are integral membrane proteins, which bind homotypically to claudin on adjacent endothelial cells to form the primary seal of the tight junctions. Claudin-1, -2, -3, -5 and -11 are expressed in the brain and especially claudin-11 is presented in oligodendrocytes myelin sheaths (Furuse *et al.*, 1998; Morita *et al.*, 1999; Wolburg *et al.*, 2003; Lee *et al.*, 2003; Liebner et al., 2000). Among them, claudin-1, -3, and -5 are mainly associated with maintenance of normal BBB function in brain vessels (Wolburg *et al.*, 2003; Lee *et al.*, 2003; Liebner *et al.*, 2000). In knockout mice of claudin-5, the development and morphology of BBB tight junction are not altered but show a selective increase in paracellular permeability of small molecules (Nitta *et al.*, 2003; Matter and Balda, 2003). These investigations suggest that the composition of claudin species directly determines BBB function.

JAMs are localized at the tight junctions and are members of the immunoglobulin superfamily, and have a role in junction assembly, leukocyte transmigration and platelet activation (Martin-Padura *et al.*, 1998; Bazzoni,

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2003). Appearance of JAMs often correlates with functional changes of junctions, such as reduced paracellular permeability and enhanced electrical resistance (Bazzoni, 2003).

Tight junctions are also made up of several accessory proteins that are necessary for structural support. Zonula occludens proteins-1 and -2 (ZO-1 and ZO-2) bind directly to actin filaments, suggesting that these molecules function as cross-linkers between tight junction strands and actin filaments (Wolburg and Lippoldt, 2002; Fanning *et al.*, 1998). ZO-2 has been reported to associate with ZO-1, and ZO-3 also associates with ZO-1, but not with ZO-2 (Fanning *et al.*, 1998; Haskins *et al.*, 1998). ZO-1, ZO-2 and ZO-3 directly bind to claudin and also bind to the cytoplasmic domain of occludin (Haskins *et al.*, 1998; Itoh *et al.*, 1999a). Occludin in tight junction strands may also be cross-linked to JAMs by ZO-1 (Itoh *et al.*, 1999b). The peripherally tight junction associated proteins 7H6 and cingulin were also found in the BBB (Citi *et al.*, 1988; Zhong *et al.*, 1993). Tight junctions are important in the BBB regulation during many physiological and pathological insults, but the exact role of these tight junction proteins in the BBB permeability control has not fully understood.

Although the tight junction ultimately determines the barrier properties of endothelium, the adherens junction mediates the initial adhesion between endothelial cells (Staddon and Rubin, 1996) (Fig. 1). The endothelial specific vascular endothelial-cadherin (VE-cadherin) is an important determinant of microvascular integrity both *in vitro* and *in vivo* (Corada *et al.*, 1999; Minagar *et al.*, 2003). VE-cadherin has been shown to be downregulated in brain microvessels late during brain angiogenesis (Breier *et al.*, 1996). VE-cadherin is linked to the cytoskeleton *via* cytoplasmic tail of catenins, and this complex acts as an early-recognition component between endothelial cells (Lampugnani *et al.*, 1995).

The tight junctions in the BBB prevent significant passive movements of small hydrophilic molecules like sodium, hydrogen, bicarbonate and other ions from the blood to the brain. But there are many specialized transport systems on the endothelial cells, astrocytes and neuronal cells. They mediate the direct transport of essential

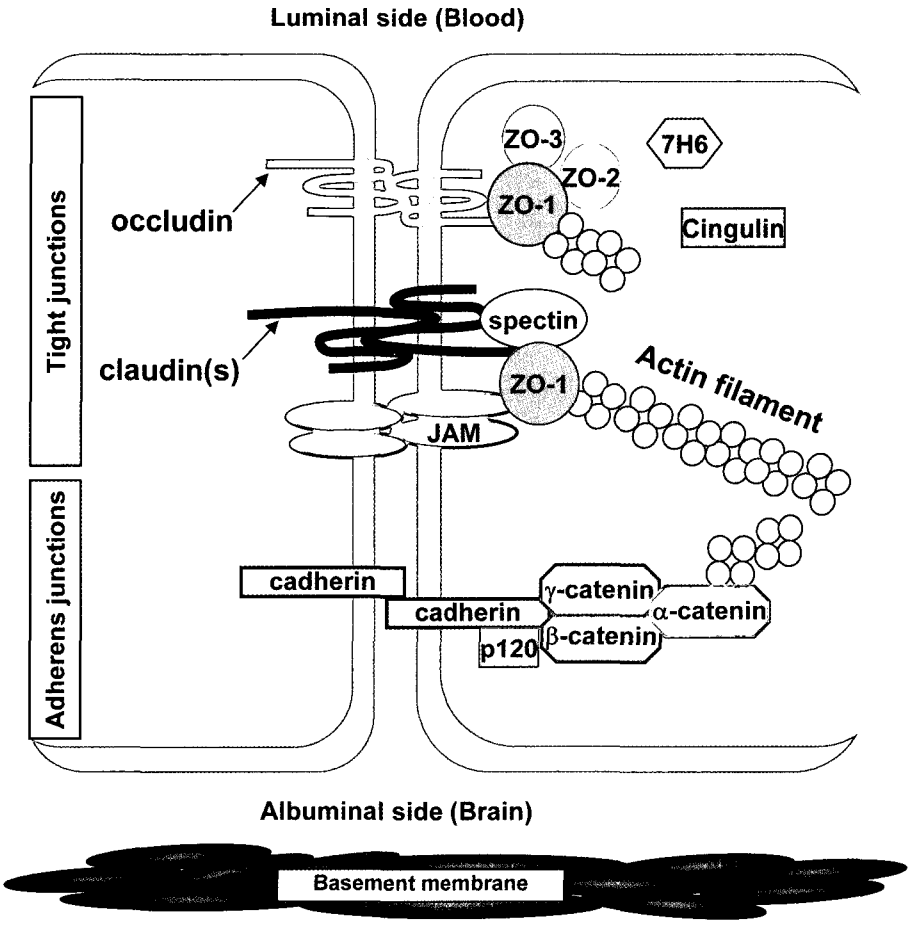

Fig. 1. Molecular structure of tight and adherens junctions. The transmembrane proteins mediate the contact between endothelial cells, and bind to the actin cytoskeleton via cytoplasmic junctional proteins. JAM, junctional adhesion molecules; ZO, zonula occludens protein.

substances such as glucose, amino acids, thyroid hormones and other nutrients into the CNS or of toxic metabolites out of the CNS (Demeule *et al.*, 2002).

Glucose transport systems in the brain are important because glucose is the crucial metabolic substance in the brain. Glucose transporter-1 (Glut-1) is expressed in many cell types, with exceptionally high abundance in tissues with barrier function, such as brain microvessels and retinal capillaries (Duelli and Kuschinsky, 2001; Harik *et al.*, 1990). Glut-1 regulates the transport of glucose from blood to the brain and there are two kinds of Glut-1, 55 kDa and 45 kDa forms. The 55 kDa form is expressed in microvascular endothelium of the CNS, commonly related to the BBB (Harik *et al.*, 1990; Pardridge *et al.*, 1990). In contrast, the 45 kDa Glut-1 is not found in brain microvasculature even though constitutes the greatest proportion of total Glut-1 protein presenting in the adult brain. Generally, 45kDa Glut-1 is expressed in neuronal and glial cells in the brain (Walker *et al.*, 1988; Maher *et al.*, 1994). The specific appearance of Glut-3 in neurons suggests a role in neuronal maturation, which includes the metabolic supply for dendritic and axonal traffic (Nagamatsu *et al.*, 1992).

P-glycoprotein, the product of the multidrug resistance gene, is also one of the important systems in the BBB. It actively transports a variety of small molecules out of the brain (Demeule *et al.*, 2002). P-glycoprotein is expressed in only limited tissues with barrier function including epithelia of the liver, kidney, small and large intestine, and capillary endothelial cells in the brain, ovary, and testis. The expression of P-glycoprotein is detected in BBB endothelial cells of human and rat, suggesting that P-glycoprotein may have a general function in the mammalian BBB (Cordon-Cardo *et al.*, 1989; Thiebaut *et al.*, 1989).

Aquaporins (AQPs) are recently discovered as water transporting proteins expressed in many mammalian epithelia and endothelia (Verkman *et al.*, 2002). AQP1 is strongly expressed in most microvascular endothelial cells in the cornea and intestine. AQP1 is also expressed in choroids plexus epithelia in the brain but not in brain vessels, so it may be important in the formation of cerebrospinal fluid (Verkman and Mitra, 2001). Although AQPs have not been identified in endothelial cells in the brain, AQP4 is strongly expressed in astrocyte foot processes and tightly associated with the BBB maintenance (Verkman *et al.*, 2002; Verkman and Mitra, 2001).

Even though the characteristics of the BBB are well defined, the mechanisms of development, differentiation and maintenance of the BBB have little known. Understanding the mechanisms of development and maintenance of the BBB is important because many brain diseases such as brain tumors are closely related with the BBB dysfunction. This review will focus on a possible molecular mechanism of the BBB differentiation mediated by oxygen tension and growth factors, and on the molecular mechanisms of BBB disruption observed in brain tumors.

DEVELOPMENT OF THE BBB: THE MOLECULAR AND CELLULAR MECHANISMS

During embryonic development, the vascular network is formed by both vasculogenesis (new vessel formation) and angiogenesis (sprouting from pre-existing vessel) (Risau and Wolburg, 1990; Folkman, 2003). The development of the CNS vasculature begins via vasculogenesis. When angioblasts originated from the mesoderm migrate into the head region, they form the perineural vascular plexus that cover the brain (embryonic day 2 (E2) in chick and E9 in rodent) (Engelhardt, 2003; Plate, 1999). Next, vascular sprouts from the perineural plexus invade the avascular neuroectoderm and form the vasculature within the CNS (E4.5 in chick and E11.5 in rat) (Plate, 1999). This brain angiogenesis is maximal in the early postnatal period [up to postnatal day 10 (P10) in rat] and down-regulated in the adult (Plate, 1999).

During the late embryonic and early postnatal periods, vascular sprouting is followed by vessel differentiation and remodeling that are characterized by astrocytes and/or pericyte recruitment and formation of contacts with vessels. The differentiation of the BBB, called barriergenesis, is the final step of the brain vasculature (Engelhardt, 2003; Janzer and Raff, 1987; Rieckmann and Engelhardt, 2003). In the rat brain, angiogenesis and vascular remodeling are completed around P20 (Plate, 1999; Breier *et al.*, 1992). The two steps of BBB development, brain angiogenesis and barriergenesis, might be regulated by oxygen tension, since oxygen tension has a crucial role in the vascular development of several tissues and organs including the brain (Risau, 1997; Maltepe and Simon, 1998; Lee *et al.*, 2001).

Hypoxia and regulation of brain angiogenesis

Hypoxia, low oxygen tension, is a well-known signal for angiogenesis in many physiological and pathological processes (Bunn and Poyton, 1996). There are some previous reports that local hypoxia is present and is likely to play an important role in the vasculature development during embryogenesis (Maltepe and Simon, 1998; Lee *et al.,* 2001). Additional reports suggest that tissue oxygen tension regulates trophoblast differentiation during human placental development and retina development in monkey and rat (Genbacev *et al.*, 1997; Zhang *et al.*, 1999; Sandercoe *et al.*, 2003).

It is well established that vascular endothelial growth factor (VEGF) is increased by hypoxia and acts as an endothelial cell mitogen and potent vascular permeability

factor (Ferrara and Henzel, 1989; Yancopoulos *et al.*, 2000). VEGF is released from the ventricular layer in the developing neuroectoderm, and then induces migration and proliferation of endothelial cells from the perineural vascular plexus (Millauer, 1993). Heterozygous VEGF-deficient mice die early in the embryonic development (Carmeliet *et al.*, 1996). Disruption of the VEGFR-1 *(Flt-1)* gene results in abnormal and disorganized blood vessels. Interference of VEGFR-2 *(Flk-1)* inhibits endothelial cell differentiation, while inactivation of VEGFR-3 *(Flt-4)* leads to defective remodeling of vascular networks (Yancopoulos *et al.*, 2000; Maisonpierre *et al.*, 1997). Several studies show the expression of VEGF in rat brain is upregulated under hypoxic conditions (Nico *et al.*, 1999; Abbott, 2002). In addition, the expression patterns of VEGF and HIF-1α proteins spatiotemporally coincide in embryos including the brain (Lee *et al.*, 2001; Song *et al.*, 2005). VEGF expression is markedly decreased in hypoxic HIF-1$\alpha^{-/-}$ embryonic stem cells (Iyer *et al.*,1998). These reports indicate that VEGF is upregulated by hypoxia, and its receptors are required for the formation, remodeling and survival of embryonic blood vessels.

Putative mechanism of barriergenesis: astrocytes and molecular factors

Brain capillaries are gradually differentiated and remodeled into the BBB with impermeable properties. Interestingly, the expression of ZO-1 is developmentally regulated; high in newborn mice, the highest at P7, and then maintained in adult mice. Occludin expression is also increased after birth, and then reaches the level of the mature BBB at P14 (Hirase *et al.*, 1997; Nico *et al.*, 1999). Although it was known that inductive signals within the embryonic neural tube are responsible for committing the endothelium to barrier formation, inductive molecules and their receptors are still unidentified.

Astrocytes and their precursors have been implicated in the induction of the BBB (Janzer and Raff, 1987). Vascular ensheathment by astrocyte end-feets is unique feature of CNS capillaries and forms around the same time as the permeability barrier develops. In many *in vitro* BBB models, primary astrocytes can induce BBB characteristics such as high electrical resistance and reduced paracellular permeability when cocultured with cerebral brain endothelial cells (Abbott, 2002; Gaillard *et al.*, 2001).

Whereas molecular mechanisms between endothelial cells and astroglial cells in the developing CNS are not fully understood, several molecules involved in perivascular-mediated barrier formation in brain endothelium have been identified. For instance, glial cell line-derived neurotrophic factor (GDNF) is involved in BBB formation of brain endothelial cell (Igarashi *et al.*, 1999; Utsumi *et al.*, 2000). TGF-β produced by astrocytes downregulates the fibrinolytic enzyme tissue plasminogen activator and anticoagulant thrombomodulin of endothelial cells (Tran *et al.*, 1999). VE-cadherin promotes the interaction between endothelial cells, whereas N-cadherin located in albuminal sides may be responsible for the anchorage to other N-cadherin expressing cells such as astrocytes (Staddon and Rubon, 1996; Schnadelbach *et al.*, 2000).

An interesting correlation exists between astroglial differentiation and BBB maturation. Leukemia inhibitory factor (LIF) increases the GFAP-positive astroytes (Richards, 1996). Moreover, endothelial cells induce the differentiation of astrocyte precursor cells into astrocytes. In addition, astrocyte differentiation is prevented by a neutralizing anti-LIF antibody (Mi *et al.*, 2001), indicating that endothelial cells may contribute to differentiation of astrocytes, in part, by secreting LIF. Furthermore, recent studies have shown that FGF-2 and FGF-5 play an important role in the regulation of astroglial differentiation and BBB integrity (Reuss *et al.*, 2003). In FGF-$2^{-/-}$/FGF-$5^{-/-}$ double mutant mice brain, GFAP-immunoreactivity is reduced. Moreover, occludin and ZO-1 are reduced in blood vessels of FGF-$2^{-/-}$/FGF-$5^{-/-}$ double mutant mice. FGF-2 is synthesized in astrocytes, which expresses FGF receptors. FGF-5 is located in neurons and probably affects on astrocyte differentiation via paracrine actions.

In addition, hypoxia/reoxygenation might be closely involved in the BBB development (Song *et al.*, 2002). The disappearance of hypoxic regions and the decreased expression of VEGF are observed in developing rat brain. These findings suggest that oxygen gradient is derived by blood supply from newly developed vessels, and could lead to reoxygenation in the brain tissues. It has been shown that vascular permeability and angiogenic activity are decreased but occludin is increased by the reoxygenated astrocytes. The expressions of VEGF is reduced, whereas the expression of Ang-1 and thrombospondin-1 is increased in reoxygenated astrocytes. Therefore, reoxygenation of astrocytes may affect on the BBB maturation during the development through oxygen-regulated genes (Song *et al.*, 2002).

Src-suppressed C kinase substrate (SSeCKS) was identified as a factor that induces the BBB function (Lee *et al.*, 2003; Rieckmann and Engelhardt, 2003). SSeCKS and its human ortholog, gravin, belong to a family of scaffolding proteins that are also potential tumor suppressors. SSeCKS acts as both an anchoring protein and a substrate for PKC (Gelman *et al.*, 2002). SSeCKS is strongly upregulated in astrocytes under reoxygenation. This factor markedly reduces the expression of VEGF and stimulates astrocytic expression of Ang-1. Moreover, conditioned media from SSeCKS-overexpressing astrocytes reduce the angiogenic activities of endothelial cells both *in vitro* and *in vivo*, and decrease [^{3}H]sucrose permeability.

Conditioned media from SSeCKS-overexpressing astrocytes markedly increase and induce linear distribution of claudin-1 and ZO-1 in the cell surface (Lee *et al.*, 2003). These findings strongly indicate that SSeCKS triggers vessel maturation and stabilization during BBB development by inhibiting brain angiogenesis, on the contrary, inducing barriergenesis *via* the upregulation of tight junction proteins.

Pericytes/Neurons/Endothelial interaction in BBB remodeling

Although astrocytes are important in the differentiation of the BBB, it is likely that astrocytes are not sufficient to induce the barrier. Because some of BBB characteristics in brain endothelial cells appear very early prior to astrocyte differentiation, also iris vessels are impermeable to dyes, even in the absence of astrocytes (Engelhardt, 2003; Small *et al.*, 1993).

Pericytes are another cell population found in close association with endothelial cells, even at early stages of development, and seem to be more prevalent on neural capillaries than on other capillaries (Dehouck *et al.*, 1997). In peripheral tissues, pericytes have been reported to be involved in a variety of endothelial-specific functions, including modulation of endothelial permeability, stabilization of microvessel walls, and promotion of angiogenic processes as well as capillary sprouting (Ramsauer *et al.*, 2002). Endothelial cells form capillary-like structures in co-culture with astrocytes, and then, pericytes stabilize the capillary-like structure by preventing apoptosis of endothelium (Ramsauer *et al.*, 2002). The interaction of pericyte and brain endothelial cell are mediated by endothelial cell-secreted endothelin-1 (Dehouck *et al.*, 1997). Several factors such as Ang-1/Tie-2, PDGF-B and PDGF receptor-β and adhesion molecules such as *N*-cadherin are also required for proper endothelial cell-pericyte interaction (Lindahl *et al.*, 1997). Pericytes express PDGF receptor-β and respond to PDGF that plays a critical role in the recruitment of pericytes to newly formed vessels. Disruption of the PDGF-B gene in mice shows microvascular pericytes loss and abnormal development of capillaries (Lindahl *et al.*, 1997). Endothelium-specific PDGF-B knockout leads to reduction of pericyte density and cause vascular abnormality, indicating that pericytes regulate microvessel structure (Enge *et al.*, 2002).

Interestingly, it has been reported the synergic effects of neurons and astrocytes on the BBB differentiation. Neuron modulates the expression and localization of occludin in brain endothelial cells (Savettieri *et al.*, 2000; Schiera *et al.*, 2003). αV-integrin knock-out mice develop intracerebral hemorrhages and abnormal vasculature (McCarty *et al.*, 2002). Normal recruitment and association of pericytes with endothelial cells are observed in brain vessels of αV-integrin knock-out mice. However, the defective associations between cerebral microvessels and brain parenchymal cells such as neuroepithelial cells, glia and neuronal precursors are detected (McCarty *et al.*, 2002). It has been reported that proliferating neuronal cells are found in dense clusters associated with the vasculature, and roughly 37% of all dividing cells are immunoreactive for endothelial markers (Palmer *et al.*, 2000). This result suggests that neurogenesis is intimately associated with active vascular recruitment and subsequent remodeling. In addition, a possible involvement of neuronal precursor cells in inducing BBB characteristics of immature endothelial cells has also been suggested (Bauer and Bauer, 2000).

Brain tumors and BBB

Angiogenesis of brain tumors

The most common primary tumors arising in the CNS are gliomas, consisting of glioblastoma, astrocytomas, oligodendrogliomas and ependymomas (Kleihues and Cavanee, 2000; Collins, 2002). High-grade gliomas are characterized by extensive microvascular proliferation showing a higher degree of vascularity than low-grade gliomas and normal brain. The degree of neovascularization in high-grade gliomas is histological indicators of the degree of malignancy and prognosis of patients. Like other solid tumors, glioblastomas have extensive hypoxic regions and necrosis. Recent report indicates that the progression of gliomas is the response of tumor cell to low-oxygen conditions, which is elicited via the stabilization and activation of HIF-1α (Kaur *et al.*, 2005).

Glioblastoma multiforme (GBM) is the most aggressive form of gliomas and can occur as the result of progression from lower grade astrocytomas. For example, pathological examination of low-grade astrocytomas (WHO grade II) demonstrates the diffusely infiltrating tumor cells in the normal brain, which begins as nonangiogenic tumors that have the ability to co-opt blood supply from existing vasculature. When grade II astrocytomas progress to grade III (anaplastic astrocytomas), tumor cell densities are increased and vascular density is also mildly increased. The most dramatic histologic changes are seen in the transition to GBM (grade IV). There are extreme levels of microvascular hyperplasia in regions adjacent to necrosis and more widespread invasion and multiple hypoxic regions in the growing periphery of GBM. Hypoxic induction of VEGF is considered to be a major driving force behind new vessel development in gliomas. The production of VEGF in gliomas is significant, i.e., VEGF levels in the cyst fluid of GBM patients are 200- to 300-fold higher than those present in the serum (Takano *et al.*, 1996).

Disruption of the BBB in brain tumors

Dysfunction of the BBB is found in brain tumors (Fig. 2). The pathological aspects of the BBB involve the cumulative effects of multiple factors such as pro-inflammatory mediators, vasoactive cytokines and growth factors, which are derived from both the glia and endothelium (Petty and Wettstein, 2001; Nag, 2002). These released factors cause the disruption of BBB tight junction, which increases the permeability between adjacent endothelial cells and consequently leads to vasogenic edema and hemorrhage (Huber *et al.*, 2001).

Many brain tumors cause complete breakdown of the BBB and leak fluid into the brain, this leads to peritumoral edema. The low-grade astrocytomas may contain vessels with close-to-normal barrier characteristics. In contrast, malignant primary tumors (i.e., high-grade astrocytoma, glioblastoma) and brain metastases of systemic cancers (i.e., metastatic adenocarcinoma) contain vessels that are excessively leaky and lack differentiated transport properties of normal BBB vessels (Kandel *et al.*, 2000). It has been reported that claudin-1 expression is lost from nearly all microvessels in high-grade astrocytomas. In contrast, claudin-5 and occludin expression are only down regulated in hyperplastic vessels, indicating that claudin-1 down-regulation is an early marker of barrier dysfunction (Liebner *et al.*, 2000). Occludin expression shows an inverse correlation by increasing malignancy of adult human non-neoplastic brain tumors (Papadopoulos *et al.*, 2001). In addition, selective loss of claudin-3 immunostaining has been observed in cerebral microvessels of human GBM (Matter and Balda, 2003).

The abnormal properties of tumor endothelial cells are presumably explained either by the absence of normal interactions between astrocytes and capillaries or by the secreting factors from tumor cells. In high-grade astrocytomas, undifferentiated neoplastic astrocytes do not produce factors necessary to maintain the BBB in the tumor region. VEGF produced by astrocytomas may account for the excessive proliferation and permeability of the glioblastoma vessel (Takano *et al.*, 1996; Kandel *et al.*, 2000). The expression of VEGF receptor is stimulated in astrocytoma microvessels with increasing malignancy (Plate *et al.*, 1994; Valter *et al.*, 1999). Moreover, VEGF decreases the expression of occludin and disrupts ZO-1 and occludin organization in cell boundary (Wang *et al.*, 2001). VEGF rapidly increases occludin phosphorylation as well as the tyrosine phosphorylation of ZO-1, which leads to tight junction disassembly and increases permeability (Antonetti *et al.*, 1999).

Brain tumor metastasis usually results from the spread of neoplastic cells in the bloodstream rather than from overgrowth of a local primary malignant tumor (Fried and Buckley, 1930). Tumor cells break the endothelial cell layer and spread through the vessels, especially in their basal lamina. Besides the loss of BBB characteristics in tumor vessels, a different composition of the basement membrane has been observed (Rascher *et al.*, 2002). The tumor vessels lacking occludin, claudin-5 and claudin-1 also showed that agrin is absent from basal lamina of GBM vessels.

The abnormal vessel permeability in brain tumors accounts for excessive accumulation of interstitial fluid. The expression of AQP1 is increased in astrocytomas while there is little AQP1 immunoreactivity in normal brain

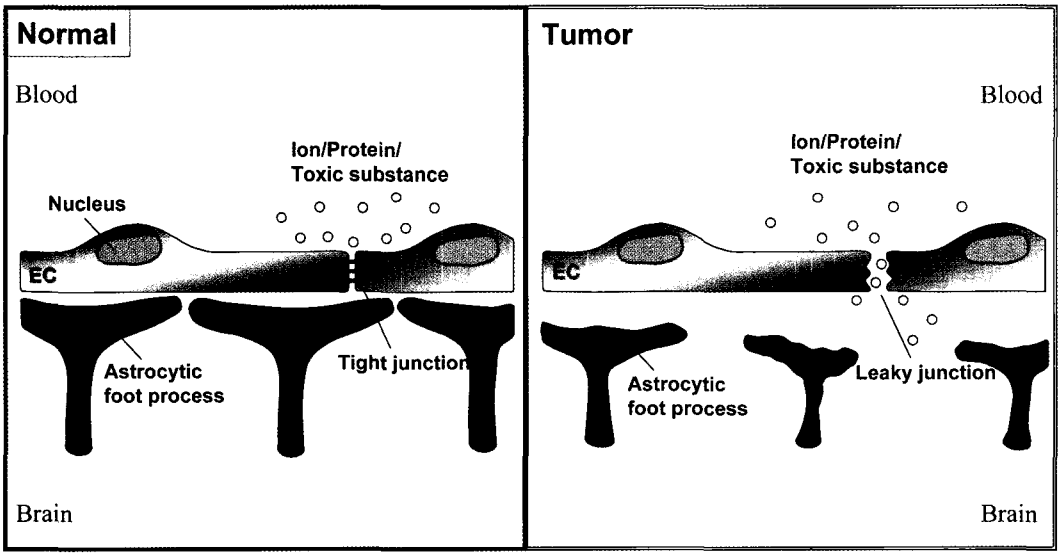

Fig. 2. Astroglial-endothelial interaction under normal and tumor conditions. In normal condition, the BBB is formed by capillary endothelial cells surrounded by astrocytic perivascular endfeet. The barrier protects the brain from ion, protein, and toxic substances. In the tumor condition, the barrier is broken down and toxic substances are penetrated easily into the brain, which might lead to the disruption of brain function.

parenchyma (Saadoun *et al.*, 2002). Also, alterations in AQP4 expression are associated with the disruption of brain homeostasis. AQP4 expression is upregulated in high-grade astrocytoma and metastatic adenocarcinoma (Saadoun *et al.*, 2002). The focal ischemia follows AQP4 mRNA upregulation in BBB disrupted regions. When siRNA to specifically suppress AQP4 expression was used, ischemia-induced cerebral edema was reduced (Nicchia *et al.*, 2003). There is significant correlation between AQP4 expression and the degree of cerebral edema, but it is unclear whether the increased AQP4 expression enhances or clears the edema formation. However, AQP4 knock-out mice show less cerebral edema and better clinical outcome than controls, following middle cerebral artery occlusion, suggesting that AQP4 inhibition may be a new therapeutic mechanism for reducing cerebral water accumulation in brain tumors (Manley *et al.*, 2000).

Brain uses glucose as its major energy source; this glucose crosses the BBB by Glut-1-mediated diffusion. Autopsied human brain tumors and experimental brain neoplasms have revealed that Glut-1 expression in brain tumor microvessels is inversely related to the degree of tumor malignancy (Guerin *et al.*, 1990). Glut-1 is expressed in endothelial cells of blood vessels of the peritumoral region and in microvessels around metastasis, but was absent in tumor microvessels and within the metastasis (Guerin *et al.*, 1990; Zhang and Olsson, 1997). These results showed that functional changes regarding glucose transport occur within metastastic tumor rather than in the peritumoral region, Therefore, Glut-1 may be considered a useful marker for brain tumor metastasis.

Dysfunction of the BBB is the cause of brain tumor expansion and metastasis; however, an essential cause of chemotherapy's low efficacy is insufficient drug delivery to the tumor site due to the presence of the BBB. Therefore, a deeper understanding of the BBB is vital for both verification of brain tumor pathogenesis and the development of methods to bypass the BBB or to increase its permeability to anticancer drugs.

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