Basic fibroblast growth factor opens and closes the endothelial blood-brain barrier in a concentration-dependent manner

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Abstract

Multiple paracrine factors have been implicated in the regulation of barrier properties of human brain endothelial cells (BECs) in different physiologic and pathologic settings. We have recently demonstrated that autocrine secretion of basic fibroblast growth factor (bFGF) by BECs is necessary for the establishment of endothelial barrier (as demonstrated by high trans-endothelial electric resistance, TEER), whereas exogenous bFGF inhibits TEER in a concentration-dependent manner. In the present study we analysed the contribution of MAPK/ERK and STAT3 signalling pathways to the inhibitory effects of exogenous bFGF. Treatment with bFGF (8 ng/ml) for 3 days increased phosphorylation of ERK1/2 and STAT3. Treatment with FGF receptor 1 (FGFR1) inhibitor PD173074 (15 μM) suppressed both basal and bFGF-induced activation of ERK1/2 and STAT3. Suppression of STAT signalling with Janus kinase inhibitor JAKi (15 nM) alone or in the presence of bFGF did not change TEER in BEC monolayers. Exposure to JAKi affected neither proliferation, nor expression and distribution of tight junction (TJ) proteins claudin-5, occludin and zonula occludens-1 (ZO-1). In contrast, treatment with MEK 1/2 inhibitor U0126 (10 μM) partially neutralized inhibitory effect of bFGF thus increasing TEER, whereas U0126 alone did not affect resistance of endothelial barrier. Our findings demonstrate that MAPK/ERK signalling pathway does not affect autocrine bFGF signalling-dependent BECs barrier function but is largely responsible for the disruptive effects of the exogenous bFGF. We speculate that bFGF may (depending on concentration and possibly origin) dynamically regulate permeability of the endothelial blood-brain barrier.

Introduction

The blood–brain barrier (BBB), that governs selective exchange of substances between blood and the parenchyma of the central nervous system (CNS), is formed by the continuous layer of specialised brain endothelial cells (BECs), clasped by tight and adherent junctions (TJs and AJs, respectively) [1]. Together with adjacent pericytes, smooth muscle cells, astrocytes and neurones, BECs are integrated into complex multi-cellular structure of neurogliovascular unit (NGVU) involved in the precise coordination of local blood flow, transport across the BBB, energy metabolism and formation of the brain active milieu [2–4]. Cellular components of the NGVU interact through multiple mechanisms, including numerous paracrine factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoetins, sonic hedgehog (SHH), retinoic acid (RA) and others [5, 6]. Identification of precise mode of action of these paracrine factors in different physiologic and pathologic settings remains challenging. Firstly, majority of currently available *in vivo* models do not allow accurate control and dynamic monitoring of local fluctuations of different paracrine factors at the BBB. Secondly, defined protocols, allowing generation of BECs monocultures with high transendothelial electrical resistance (TEER) values comparable with those recorded *in vivo*, have been introduced only relatively recently. In our previous study, which employed a fully defined differentiation protocol [7] for producing BECs from human inducible pluripotent stem cells (iPSCs), we demonstrated a dual role for bFGF in the regulation of BECs barrier function [8]. Autocrine secretion of bFGF is required for the establishment of tight-junctions

defining BECs barrier, whereas exogenous bFGF in high concentrations (> 4 ng/ml) disrupts the barrier integrity [8]. We also found that inhibitory effects of exogenous bFGF on the *in vitro* BBB depend neither on phosphoinositide 3-Kinase (PI-3K), nor on ROCK signalling pathways [8]. In the present study we further investigated molecular mechanisms of the inhibitory effects of exogenous bFGF. Binding of FGFs to the FGF receptor 1 (FGFR1) activates FGFR tyrosine kinase, which phosphorylates adaptor proteins and subsequent activation of rat sarcoma virus (Ras)-mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK), phosphoinositide 3-kinase (PI3K)-Akt, phospholypase Cγ (PLCγ), and signal transducer and activator of transcription (STAT) intracellular signalling pathways [9]. Since our previous study demonstrated that PI-3K cascade suppresses inhibitory effects of bFGF on TEER, we focused on the STAT and Ras-MAPK/ERK signalling. We found that inhibition of STAT signalling with Janus kinase inhibitor (JAKi) alone, or in the presence of bFGF did not change TEER of BEC monolayers. In contrast, treatment with MEK 1/2 inhibitor U0126 alone did not affect TEER, but significantly increased TEER values in bFGF-treated BECs, thus partially neutralising inhibitory effect of exogenous bFGF. Our findings demonstrate that MAPK/ERK signalling pathway does not affect autocrine bFGF signalling-dependent BECs barrier function but is largely responsible for the disruptive effects of the exogenous bFGF. The bFGF therefore acts as a dynamic regulator of the BBB which opens it at high and closes at low concentrations.

Methods

Maintenance of iPSCs and differentiation to the brain capillary endothelial cells (BECs)

In this study two healthy donor-derived iPSC lines, MBE 2960 (male, 78 years old) and SHED-iPSC (female, 7 years old), from the passages 12 to 34, were used. Both iPSC lines were cultured on matrigel-coated (Corning) plates with Essential 8 (E8) medium exchange every 24 hours. iPSCs were differentiated to BECs according to slightly modified previously published protocol [7]. Briefly, 24 hours after splitting, the differentiation was initiated by changing the E8 to the Essential 6 (E6) medium (Thermo Fisher Scientific). E6 was fully refreshed every 24 hours for four days. On the fifth day, the E6 was changed to the human Endothelial Serum Free-Medium (hESFM, Thermo Fisher Scientific) supplemented with 20 ng/ml bFGF (Thermo Fisher Scientific), 10 μM retinoic acid (Merck Darmstadt, Germany), and 0.25× B-27 (Thermo Fisher Scientific). After 48 hours the hESFM medium was fully refreshed. The next day BECs were split for selection onto 400 μg/ml collagen IV and 100 μg/ml fibronectin-precoated (both from Merck) Transwell inserts (Corning) or 48-well plates (TPP). After four days, BECs monolayer barrier was characterised by measuring TEER and expression of claudin-5, occludin, ZO-1, VE-cadherin, CD34, and von Willebrand factor by immunocytochemistry and Western blotting (Fig. 1).

Experimental design

All treatments of BECs were performed once by refreshing the medium on the next day after the differentiation (see Fig. 2A for experimental design). The medium administered to the different experimental groups of BECs contained one of the following: 8 ng/ml bFGF, 15 μM PD173074 (Tocris Bioscience), 15 nM JAK inhibitor I (JAKi, Santa Cruz), 10 μM U0126 (MedChemExpress), bFGF + PD173074, bFGF + JAKi, bFGF + U0126. After three days of the treatment, BECs were subjected to TEER measurement and immunocytochemistry or Western blot analysis. Additionally, in the experiment with U0126, BECs were analysed by Western blot after 2 and 24 hours of the treatment (see Fig. 4A for experimental design).

Transendothelial electrical resistance (TEER) measurements

To measure TEER, BECs were grown on Transwell inserts (Corning) with 0.4 μm pore size and 0.33 cm^2 surface area polyester membrane. TEER was measured with Millicell ERS-2 Electrical Resistance System (Merck-Millipore). Each insert was measured three times in different locations. To calculate TEER ($\Omega*cm^2$), the mean electrical resistance of the BECs-free inserts was subtracted from the mean readings of the inserts with BECs monolayers and then multiplied by the surface area of the insert.

Immunocytochemistry

Immediately after TEER measurement BECs were fixed and permeabilised by incubating them with ice-cold methanol and acetone in a 1:1 ratio at -20°C for 10 min. Then the cells were washed three times with PBS and blocked with 1% BSA-PBS solution at room temperature for 30 min. Afterwards, the primary antibodies against ZO-1 (1:33), claudin-5 (1:100) and occludin (1:50, all from Thermo Fisher Scientific) were diluted in blocking solution and administered to the cells for incubation at 4°C overnight. Next, cells were washed three times with PBS and incubated with the secondary antibodies conjugated with Alexa Fluor 594 (1:1000, Thermo Fisher Scientific) diluted in PBS at room temperature, in the dark, for 1 hour. Then the samples were washed three times and mounted on coverslips using an aqueous fluorescent mounting medium with DAPI (Abcam). Prepared samples were analysed with the Leica TCS SP8 confocal microscope (Leica Microsystems, Mannheim, Germany) using lasers of Diode 405 nm and DPSS 561 nm and 63x oil immersion lens.

The images of confocal microscopy were used to cell count evaluation. Cells were counted according to the DAPI stain using the ImageJ program, a multi-point tool.

Western blot

Cells growing in a 48-well plate were washed three times with cold PBS and incubated with RIPA buffer (Thermo Fisher Scientific) containing 1× protease inhibitor cocktail (Thermo Fisher Scientific) on ice for 15 min. Obtained lysates were collected, vortexed and placed on ice again for the next 10 min. Afterwards, the samples were centrifuged at 18 000 *g* at 4°C for 20 min and the remaining supernatants were collected to new tubes. The concentration of proteins was measured with the NanoPhotometer Pearl

(Implen) and equalised between samples with 1x Laemmli sample buffer (Bio-Rad). Then lysates were diluted in 6x Laemmli sample buffer and denatured at 95°C for 5 min. Protein lysates were subjected to 4–10% polyacrylamide gel electrophoresis using Mini-PROTEAN Tetra cell apparatus (Bio-Rad). Separated proteins were blotted onto a polyvinylidene difluoride membrane by a semidry Trans-Blot Turbo transfer apparatus (Bio-Rad). The membrane was blocked with 5% bovine serum albumin (Applichem) diluted in PBS with 0.18% Tween-20 (hereinafter referred to as PBS-T) at room temperature on a platform rocker for 1 h. The primary antibodies against pAkt Ser473 (1:2000), pAkt Thr308 (1:1000), pERK (1:2000), pSTAT3 Ser727 (1:1000), pSTAT3 Tyr705 (1:800), β-actin (1:1000, all from Cell Signaling Technology) and α-tubulin (1:500, Thermo Fisher Scientific) were diluted in a blocking solution and the membrane was then probed with them and incubated at 4°C overnight. Then the membrane was washed three times with PBS-T on a platform rocker for 5 min and incubated with horseradish peroxidase-conjugated secondary antibodies (1:2000, Thermo Fisher Scientific) diluted in PBS-T on a platform rocker at room temperature for 1 h. Washing procedure was repeated and immunoreactive bands were detected with Clarity ECL Western blotting substrate (Bio-Rad) using ChemiDoc MP system (Bio-Rad).

Statistics

Statistical analysis was performed from at least three biological experiments using Graph Pad Prism® software 8.0.2 (Graph Pad Software, Inc., City, State, USA). Data in the graphs is presented as the mean ± standard deviation (± SD). Differences between the groups were compared by one-way ANOVA following Tukey's post-test. Results were considered significant at $p < 0.05$.

Results

Exogenous bFGF activates ERK 1/2, STAT3 and Akt signalling pathways in differentiated BECs

We demonstrated previously that exogenous bFGF at concentrations above 4 ng/ml inhibits TEER and proliferation of BECs in a concentration-dependent manner [8]. After bFGF binding, FGF receptor 1 (FGFR1) activates several downstream signalling pathways, including Ras-MAPK, PI-3K-Akt and STAT [9]. We treated differentiated BECs with 8 ng/ml of exogenous bFGF for 3 days and monitored levels of phosphorylation of ERK 1/2, Akt (Thr308/Ser473) and STAT3 (Ser727/Tyr705) proteins. We found that treatment with bFGF significantly increased phosphorylation of ERK 1/2 and STAT3 (Ser727) and slightly increased of phosphorylation Akt at Thr308 (Fig. 2B, C). Combined treatment with selective inhibitor of FGFR1 PD173074 (15 μM) almost completely suppressed phosphorylation of ERK 1/2 and STAT3 at Ser727 and Tyr705 but not of Akt (Fig. 2B, C). Treatment with PD173074 alone strongly suppressed ERK 1/2 and STAT3 (Tyr705) phosphorylation (Fig. 2B, C) highlighting the importance of autocrine/paracrine bFGF signalling for maintaining the basal activity of ERK 1/2 and STAT3 in differentiated BECs. Inhibition of FGFR1 with PD173074 did not affect Akt phosphorylation (Fig. 2B, C).

Inhibition of JAK/STAT signalling does not affect bFGF-mediated suppression of TEER in BEC monolayers

We used JAKi (15 nM), a potent inhibitor of janus kinases (JAK) [10] to block STAT signalling in differentiated BECs (Suppl. Figure 1). Treatment of BECs monolayers with JAKi alone affected neither TEER nor proliferation of BECs (Fig. 3A, B), whereas bFGF administered on its own down-regulated TEER by 89.53 ± 7.77% ($n = 4$, $p < 0.0001$, Fig. 3A) and increased cell numbers by 90.00 ± 31.61% ($n = 4$, $p = 0.008$, Fig. 3B). JAKi did not affect bFGF-mediated suppression of TEER and increase of BCEC proliferation (Fig. 3A, B). Neither did it alter expression and distribution of TJ proteins claudin-5, occludin and ZO-1 (Fig. 3C).

Inhibition of ERK 1/2 signalling partially neutralised bFGF-mediated suppression of TEER in BEC monolayers

Treatment with exogenous bFGF (8 ng/ml) induced long-term activation of ERK 1/2 signalling in the differentiated BECs (Fig. 4). ERK 1/2 phosphorylation was effectively suppressed by U0126 (10 μM), a potent inhibitor of MEK 1/2 kinases, upstream activators of ERK 1/2 (Fig. 4B, C). We found that U0126 (10 μM) partially neutralised inhibitory effect of bFGF by increasing TEER by 40.95 ± 23.49% ($n = 4$, $p = 0.0467$), whereas U0126 alone did not affect barrier properties of BECs (Fig. 5A). Treatment with U0126 alone or in combination with bFGF did not affect BEC proliferation and expression/distribution of TJ proteins (Fig. 5B, C). At the same time, U0126 suppressed STAT3 (Ser727) phosphorylation in bFGF-treated and untreated BECs (Suppl. Figure 1) indicating that Ser727 represents a downstream target for ERK 1/2.

Discussion

As we discovered before, autocrine secretion of bFGF by BECs is necessary for the establishment of proper barrier function, whereas exogenous bFGF in concentrations exceeding 4 ng/ml inhibits TEER of BECs monolayers in a concentration-dependent manner [8]. In this study we analysed the role of intracellular MAPK/ERK and STAT signalling pathways in mediating FGFR effects [9], in particular the contribution of these cascades to the inhibitory action of the exogenous bFGF on the barrier function of BECs. We found that inhibition of bFGF autocrine/paracrine signalling with selective inhibitor of FGFR1 PD173074 completely suppressed basal ERK 1/2 phosphorylation in differentiated BECs (Fig. 2B, C). The basal ERK 1/2 activity, however, was not required for the establishment of BECs barrier, because inhibition of ERK 1/2 phosphorylation with U0126 did not change TEER (Fig. 5A). We therefore conclude that ERK 1/2 signalling pathway does not affect autocrine bFGF regulation of BEC barrier.

We also found that PD173074 decreased both basal and bFGF-induced activity of STAT3 (Fig. 2B, C). In this study we used antibodies against phospho-STAT3 (Ser727/Tyr705). Phosphorylation of STAT3 at Ser 727 is required for its maximal transcriptional activation [11] and can be mediated by different

members of MAPK family p38 [12], ERK1/2 [13, 14], c-Jun N-terminal Kinase (JNK) [15], protein kinase C (PKC) [16], and mammalian target of rapamycin (mTOR) [17]. We found that inhibitor of MEK 1/2 kinases U0126 suppressed basal and bFGF-induced Ser727 phosphorylation in BECs (Suppl. Figure 1B). This indicates that Ser727 of STAT3 represents a downstream (direct or indirect, which remain unknown) target for ERK 1/2. As under basal conditions U0126 does not affect TEER, we concluded that STAT3 (Ser727) signalling does not contribute to autocrine bFGF regulatory effect on BECs barrier function. Similarly, inhibition of STAT signalling with JAK inhibitor did not change TEER in BECs (Fig. 3A).

In our previous report, using the same experimental conditions, we found that treatment of BEC monolayers with PI-3K inhibitor LY294002 (25 μM) down-regulates TEER by approximately 40% [8] showing that basal PI-3K-Akt signalling contributes to the regulation of the BEC barrier. However, we did not detect down-regulation of Akt phosphorylation (Fig. 2B, C) in response to the treatment with PD173074, therefore basal PI-3K-Akt signalling in BECs is not linked to the autocrine/paracrine bFGF signalling. Further studies are needed to elucidate the mechanisms involved in the regulation of autocrine bFGF signalling-dependent BEC barrier function.

Treatment with high concentration of exogenous bFGF (8 ng/ml) induced long-term activation of ERK 1/2 signalling (Fig. 4) and effectively suppressed TEER in the differentiated BECs. We also found that inhibition of ERK 1/2 with U0126 partially neutralised inhibitory effects of bFGF (Fig. 5A, B). Several studies demonstrated that MAPK/ERK signalling pathway can modulate permeability of endothelial and epithelial barriers by modulating expression and distribution of TJ proteins [18]. MAPK/ERK signalling cascades may promote or disrupt endothelial barriers in stimulus- and cell type-dependent manner [18]. For example, H_2O_2 induces paracellular permeability of porcine brain-derived microvascular endothelial cells by activating ERK 1/2 kinase pathway and these changes correlate with localisation of TJ proteins ZO-1 and ZO-2 [19]. Exposure to microwaves damages BBB through the VEGF/Flk-1-ERK-dependent Tyr phosphorylation of occludin and inhibition of its interaction with ZO-1 [20]. In our model similar mechanisms might be responsible for the inhibitory action of the exogenous bFGF. Therefore, the effects of bFGF/ERK signalling on the interaction between ZO and other TJ proteins in BECs are in need of systematic exploration in the future studies. Our finding that MEK 1/2 inhibitor U0126 suppressed FGF-induced STAT3 (Ser727) phosphorylation indicates that it represents a downstream target of ERK 1/2 which could be potentially responsible for the inhibitory action of the exogenous bFGF. Indeed, several studies demonstrated that IL-6 family cytokines promoted BBB breakdown through the activation of STAT3 signalling pathway [21, 22]. Hence, the importance ERK 1/2-STAT3 signalling axis for the inhibitory action of the exogenous bFGF should be addressed in the future studies.

We have previously reported that inhibition of FGFR1 and PI-3K signalling significantly decreased proliferation in bFGF untreated BECs and that these effects were paralleled with substantial reduction of TEER [7]. However, the present study shows that MEK 1/2 inhibitor U0126 does not affect proliferation of bFGF untreated BECs (Fig. 5B) indicating that under basal conditions BEC proliferation occurs through ERK-independent mechanisms. These findings suggest possible relationship between BEC proliferation and barrier establishment.

We did not test how PLCγ, another mediator of the intracellular FGFR signalling [9] affects BECs barrier integrity. FGFR kinase recruits and activates the PLCγ which produces inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG) by the hydrolysis of phosphatidylinositol (4,5) bisphosphate (PIP_2). $InsP_3$ induces calcium release from the intracellular stores while DAG activates DAG-sensitive protein kinases C (PKC) and protein kinases D (PKD) [23]. Some of the PLCγ downstream targets can be potentially involved in the regulation BEC barrier. For instance, acoustic wave stimulation activated calcium-dependent activation of PKC-δ pathway that mediated dissociation of ZO-1 and occludin, promoted paracellular permeability and opening of BBB [16]. Further research is needed to establish whether similar mechanisms can be responsible for the inhibitory effects of exogenous bFGF in BCECs.

Conceptually, bFGF can derive from either luminal (blood) or abluminal (parenchymal), or from both sides of the endotehlial barrier. In our experiments exogenous bFGF was added to both upper and lower compartments of the Transwell inserts and therefore the differential effects of bFGF on the luminal and abluminal sides of BEC monolayer were not distinguished. The potential differences between the effects of luminal and abluminal bFGF on the BEC barrier function should be carefully explored in the future studies.

What is the source of paracrine bFGF in the NGVU? First of all, we can not exclude possibility that BECs can increase expression and secretion of the bFGF in response to the external clues. Some indirect evidence indicates that BECs can secrete substantial amounts of bFGF that act in the paracrine manner on the surrounding tissues. For instance, tumour microvascular endothelial cells secrete bFGF which promotes cancer stem cell features in differentiated glioblastoma cells [24]. Exosomes derived from brain microvascular endothelial cells after oxygen glucose deprivation contained increased levels of bFGF [25]. An *in vitro* BBB model could be used to explore the effects of different type of stress on the expression and secretion bFGF in BECs. Pericytes represent another potential source of bFGF in the NGVU. bFGF and FGFR1 are induced in pericytes at the periinfarct areas after brain ischemia [26]. Peripheral nerve pericytes partially modify blood-nerve barrier function through the secretion of bFGF [27]. In the adult brain, bFGF is predominantly synthesised and secreted by the astrocytes [28]. However, little is known about the role of bFGF in regulation of BBB in the unperturbed adult brain [1]. In contrast, many studies demonstrated that various injuries trigger reactive astrogliosis [29] associated with increased secretion of bFGF [28, 30]. Nevertheless, the effects of local bFGF increase on the BBB integrity remain unclear. Studies allowing simultaneous *in vivo* monitoring of BBB permeability together with conditional inactivation of astrocytic bFGF release and (or) deletion of FGF receptors in BECs may reveal the role of astrocytic bFGF on BBB function. Astrocytes control permeability of the endothelial blood-brain barrier by secreting several factors such as VEGF-A, which loosens the endothelial barrier, or SHH, which stimulates barrier repair [31]. The bFGF can serve towards the same means: pulsative release of bFGF can rapidly and transiently open the barrier (acting through MAPK/ERK signalling pathway), whereas in the absence of additional input autocrine bFGF secretion restores the barrier integrity (Fig. 6).

Declarations

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Data availability statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Characterisation of BECs derived from iPSCs

A: Schematic timeline of differentiation and characterisation of BECs.

B: The scheme of TEER measurements of BECs monolayers growing on a Transwell inserts on the fourth day following the differentiation, n = 4.

C: Representative confocal microscopy images of BECs labelled with antibodies against VE-cadherin, CD34, von Willebrand factor and tight junction proteins, including claudin-5, occludin and ZO-1.

Figure 2

An exogenous bFGF induces ERK 1/2, STAT3 and Akt signalling in BECs.

A: Experimental protocol.

B: Representative Western blots of pSTAT, pAkt and pERK in the cultures of BECs.

C Quantified protein phosphorylation determined by ratio of pSTAT3, pAkt, and pERK to β-actin or a-tubulin band intensity.

Figure 3

A selective JAK inhibitor does not change bFGF-induced reduction of TEER or increased cell number in BEC cultures.

A: TEER readouts after treatment with bFGF (8ng/ml) alone or in combination with JAKi (15 nM) relative to control, n = 4.

B: Cell count in BEC cultures after treatment with bFGF (8ng/ml) alone or in combination with JAKi (15 nM) relative to control, n = 3.

C: Representative confocal microscopy images of BEC cultures stained with antibodies against claudin-5, occludin and ZO-1.

Figure 4

Selective MEK1/2 inhibitor, U0126, partially reduces bFGF-induced reduction of TEER but does not change the cell numbers in BEC cultures.

A: TEER readouts after treatment with bFGF (8ng/ml) alone or in combination with U0126 (10 mM) relative to control, n = 4.

B: Cell count in a BEC cultures after treatment with bFGF (8 ng/ml) alone or in combination with U0126 (10 μM) relative to control, n = 3.

C Representative confocal microscopy images of BECs stained with antibodies against claudin-5, occludin and ZO-1.

Figure 5

High concentration of bFGF induces long-term MAPK/ERK signalling in BECs.

A: Experimental protocol.

B: Representative Western blots of pERK after 2-hours, 1- and 3-days treatment with bFGF (8ng/ml) alone or in combination with U0126 (10 μM), n = 2-4.

C: Quantified protein phosphorylation determined by ratio of pERK1/2 to α-tubulin band intensity.

Figure 6

Potential role of autocrine and paracrine bFGF in regulation of permeability of the endothelial blood-brain barrier.

The hypothesis (which is still in need of further corroboration) posits that at the low concentrations bFGF derived from autocrine secretion up-regulates expression of tight junction proteins thus increasing the resistance of the barrier. At high concentration, bFGF derived from paracrine secretion from astrocytes or pericytes decreases expression of the tight junctional proteins (acting though MEK1/2-ERK1/2 cascade) consequently 'opening' the endothelial barrier. Thus, the very same signalling molecule can be employed for rapid opening and closing the blood-brain barrier.

Supplementary Files

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- SupplementaryFig.tif