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ORIGINAL PAPER

Resistin-Inhibited Neural Stem Cell-Derived Astrocyte Differentiation Contributes to Permeability Destruction of the Blood–Brain Barrier

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Abstract

Neuroinflammation is an important part of the development of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's and amyotrophic lateral sclerosis. Inflammatory factors destroy the balance of the microenvironment, which results in changes in neural stem cell differentiation and proliferation behaviour. However, the mechanism underlying inflammatory factor-induced NSC behavioural changes is not clear. Resistin is a proinflammatory and adipogenic factor and is involved in several human pathology processes. The neural stem cell microenvironment changes when the concentration of resistin in the brain during an inflammatory response disease increases. In the present study, we explored the effect and mechanism of resistin on the proliferation and differentiation of neural stem cells. We found that intracerebroventricular injection of resistin induced a decrease in GFAP-positive cells in mice by influencing NSC differentiation. Resistin significantly decreased TEER and increased permeability in an in vitro blood–brain barrier model, which is consistent with the results of an HBMEC-astrocyte coculture system. Resistin-inhibited astrocyte differentiation is mediated through TLR4 on neural stem cells. To our knowledge, this is the first study reporting the effect of resistin on neural stem cells. Our findings shed light on resistin-involved neural stem cell degeneration mechanisms.

Keywords Resistin · Neural stem cell · Astrocyte · Permeability · Blood–brain barrier

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Introduction

Neuroinflammation is an inflammatory response after neurodegeneration or neurological damage in the central nervous system and is an important part of the development of neurodegenerative diseases such as AD, Parkinson's and amyotrophic lateral sclerosis [1]. The initial stimulus, such as a large number of proteins aggregating, activates a sustained inflammatory and cytotoxic response and expands the secondary neuroinflammatory process, which induces changes in a patient's neurological function and the corresponding focal neurological dysfunction. Furthermore, neurodegenerative diseases are promoted by the worsening of inflammation or cytokines that pass through the blood–brain barrier (BBB) [2–4].

Neural stem cells (NSCs) are pluripotent cells that produce neuronal cells, such as neurons, astrocytes and oligodendrocytes [5]. In the adult brain, the neural stem cell pools are in the subventricular zone (SVZ) in the lateral ventricle and the subgranular layer (SGZ) of the hippocampal dentate gyrus [6–10]. The microenvironment of adult neural stem

cells is composed of tissue matrix, supporting cell populations (microglia/astrocytes), blood vessels, adhesion factors, growth factors and so on [11–15]. Under normal circumstances, neural stem cells complete their proliferation and differentiation activities in the microenvironment. However, in the disease state, inflammatory factors destroy the balance of the microenvironment, which results in changes in neural stem cell differentiation and proliferation behaviour. It is important to understand the role and mechanism of inflammatory factors' activity on neural stem cells in disease states, which is helpful for rationally interfering with the occurrence of inflammation in the brain and promoting the role of neural stem cells in the repair of disease.

Resistin is a proinflammatory and adipogenic factor. In rodents, resistin is mainly secreted by adipocytes; however, it is mainly secreted by monocytes and macrophages in humans [16]. It has been reported that resistin may play a role in obesity, insulin resistance, type II diabetes, inflammatory response, tumourigenesis and atherosclerosis [17]. As a proinflammatory factor, resistin is involved in several processes of human pathology. Studies have shown that serum resistin levels in Alzheimer's disease (AD) were significantly increased and associated with inflammatory markers such as CRP, IL-18, IL-6 and TNF-α [18, 19]. In addition, the resistin concentration in cerebrospinal fluid is positively correlated with serum concentrations in neuromyelitis optica [20]. The neural stem cell microenvironment will change when the concentration of resistin in the brain during an inflammatory response to disease increases. It is necessary to study the proliferation and differentiation effects of resistin on neural stem cells.

In this study, we investigated the effect and mechanism of resistin on the proliferation and differentiation of neural stem cells. The results will provide useful information for the repair of nervous system cells in patients with neurological inflammatory disease.

Methods

Animals and Experimental Groups

C57/BL6 mice (male) were obtained from the Lab Animal Center of China Medical University, and mice at 6–8 weeks of age and weighing 18–25 g were used in the experiments. All mice were housed in cages in a controlled environment (22–25 °C, 50% humidity). In the animal experiments on the first day, the mice were anesthetized with sodium pentobarbital (40 mg/kg). Then, resistin (250 ng in 2 µl PBS) or PBS was injected stereotactically into the lateral ventricle (AP, ± 0.34 mm; ML, ± 1 mm; DV, ± 2.25 mm). Mice were injected intraperitoneally with BrdU twice at intervals of 12 h. The mice were injected with CXCL1 or PBS with BrdU every 7 days. On the 21th day, mice were euthanized. Brain samples were collected and fixed in 4% paraformaldehyde (dissolved in 0.2 M PBS) for immunofluoresence staining.

Experimental procedures were conducted in accordance with the regulations of the animal protection laws of China and approved by the animal ethics committee of China Medical University (JYT-20060948). Efforts were made to minimize animal suffering and the number of animals used. The above protocols were reviewed and approved by the China Medical University Review Committee. All studies involving animals were performed in accordance with the animal research: reporting in vivo experiments (ARRIVE) guidelines.

Cell culture

The human brain microvascular endothelial cells (HBMECs) were obtained from surgical resections in 4- to 7-year-old children [21]. HBMECs were cultured in complete RPMI-1640 medium containing 10% foetal bovine serum (FBS), 10% Nu-serum (BD Biosciences, Franklin Lakes, NJ, USA), 2 mM glutamine, 1 mM sodium pyruvate, 1 × nonessential amino acids and 1 × MEM vitamins.

Normal and GFP-labelled mouse NSCs (Cyagen Biosciences, Guangzhou, China) were grown in DMEM/F12 (1:1) supplemented with 2% B27, 10 ng/ml EGF, 20 ng/ml bFGF and 2 mmol/l glutamine (Thermo Fisher Scientific, Shanghai, China). The cells were split every 2 days when the neurospheres had a dark clump inside or when there was ruffling on the outside of the neurospheres.

In the induced astrocyte differentiation, the 10 cm cell culture dish (Corning, Inc., Corning, NY, USA) was coated with a 1:200 dilution of Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) at 37 °C for 2 h. Next, 1×10^6 NSCs were seeded on the dish and cultured for 24 h. Then, culture medium was replaced with the astrocyte differentiation medium DMEM/F12 (1:1), supplemented with 0.5% N-2 (Thermo Fisher Scientific, Shanghai, China), 1% foetal bovine serum and 2 mmol/L glutamine. Resistin (R&D Systems, Minneapolis, MN, USA) was added to the culture medium on days 3 and 5. Samples were collected on day 14.

All the cells were maintained in a cell incubator at 37 °C, 5% CO_2 and 100% humidity.

Cell Proliferation and Cytotoxicity Assays

The proliferation of NSCs was tested using Cell Counting Kit-8 (Beyotime Biotechnology, Shanghai, China) in accordance with the manufacturer's instructions. Briefly, different concentrations of resistin were applied to NSCs for 12 h, 24 h, 36 h and 72 h. The medium was removed, and the cells were washed with PBS. Then, 90 µl of DMEM/F12 basal

medium and 10 µl of CCK-8 solution were added to each well, and the cells were cultured for another 4 h. The absorption of each well was measured using a microplate reader at a test wavelength of 450 nm and a reference wavelength of 690 nm. All the experiments were performed in triplicate. The proliferation curve was plotted with the absorbance on the ordinate versus time on the abscissa.

Flow Cytometric Analysis

The effect of resistin on the cell cycle was assessed by flow cytometry after staining the NSCs with propidium iodide (PI). Briefly, different concentrations of resistin were added to the astrocyte differentiation medium. After 24 or 72 h, cells were centrifuged and washed at 4 °C with PBS. Cells were resuspended with 300 µl PBS and fixed at 4 °C for 24 h by adding 700 µl of anhydrous ice-cold ethanol slowly (to a concentration of 70% ethanol). After centrifugation, the precipitate was washed at 4 °C with PBS. PI staining solution was added to cell precipitates and incubated at 37 °C for 30 min. The DNA content of the cells was measured using a C6 flow cytometer, and the population of each phase was determined using CFlow Plus analysis software (Accuri Cytometers Inc., Ann Arbor, MI).

Immunofluorescence

NSCs were planted on the slides coated with Matrigel (1:200) and cultured at 37 °C for 24–48 h. The cell medium was changed to astrocyte differentiation medium when the cells reached 50–60% confluence. Immunofluorescence staining was performed 7 days later. Briefly, the cells were washed with PBS and fixed with 4% paraformaldehyde for 15 min and then permeabilized with 0.05% Triton X-100 at room temperature for 5 min. After being blocked with 5% BSA at room temperature for 30 min, the cells were incubated with anti-mouse GFAP (1:1000, Proteintech, IL, USA) or anti-mouse nestin (1:250, Abcam, Cambridge, USA) antibody in 1% BSA overnight at 4 °C. After washing, all cells were incubated at room temperature for 1 h with appropriate Alexa Fluor-conjugated secondary antibodies (Invitrogen) (1:1000) in PBS. Nuclei were stained with 300 nM 4,6-diamidino-2-phenyl-indole (DAPI) for 5 min in the dark.

In other experiments, the cells were incubated with anti-mouse nestin and anti-mouse S100B (1:250, Proteintech, IL, USA) antibodies in 1% BSA overnight at 4 °C. After washing, all cells were incubated at room temperature for 1 h with Alexa Fluor-conjugated secondary antibodies (Invitrogen) (1:1000) and Fluorescent-conjugated secondary antibodies (Beyotime Biotechnology, Shanghai, China) (1:500) in PBS.

In vivo immunofluorescence assays were performed as described [22]. Briefly, mice were anaesthetized with pentobarbital (40 mg/kg) and perfused from the left ventricle with PBS to remove the blood cells, followed by 4% paraformaldehyde to fix the tissues. The mouse brain was sliced to approximately 50 microns in thickness and washed, blocked for 1 h in 8% (v/v) donkey serum (Sigma-Aldrich, Darmstadt, Germany) dissolved in 0.01 M PBS containing 0.5% Triton-X 100 (Sigma-Aldrich, Darmstadt, Germany) and incubated at 4 °C overnight with anti-GFAP antibody diluted 1:100 in 0.01 M PBS containing 0.1% Triton-X 100 and 1% (v/v) donkey serum. Alexa Fluor-conjugated IgG (Jackson Immuno Research, Hamburg, Germany), diluted 1:250 in 0.01 M PBS containing 0.3% Triton-X 100, was used to detect the specific immunostaining. Nuclei were stained with DAPI for 20 min. All the samples were covered with mounting medium (VectorLabs) and viewed via confocal microscopy (ZEN 2.1, Carl Zeiss, Germany).

RNA Interference

All siRNA sequences used in the study were designed and synthesized by Shanghai GenePharma Co., Ltd. siRNA sequences are listed in Table 1. The NSCs were transfected with non-silencing (NC) siRNA, siTLR4 (527, 1388 and 1507 are different siRNA fragments targeting TLR4 gene) or siCD36 (Table 1), and 0.75 µl Lipofectamine 3000 in 25 µl Opti-MEM after they adhered on 24-well plates. Culture medium was replaced with the astrocyte differentiation medium, supplemented with 10 or 100 ng/ml resistin, 24 h later.

Table 1 siRNA sequences

Name	Sequence	
	Sense (5′–3′)	Antisense (5′–3′)
TLR4-527	GGAUUUAUCCAGGUGUGAATT	UUCACACCUGGAUAAAUCCTT
TLR-1388	CCUACCAAGUCUCAGCUAUTT	AUAGCUGAGACUUGGUAGGTT
TLR-1507	CCAUCAUUAUGAGUGCCAATT	UUGGCACUCAUAAUGAUGGTT
CD36-1705	GACCUUACAUUGUACCUAUTT	AUAGGUACAAUGUAAGGUCTT
CD36-1324	GAUCUGAAAUCGACCUUAATT	UUAAGGUCGAUUUCAGAUCTT
CD36-758	CACAUACAGAGUUCGUUAUTT	AUAACGAACUCUGUAUGUGTT

Western Blot

Cells were lysed in RIPA buffer (Beyotime, Nantong, China) containing protease inhibitor cocktail diluted 1:25 (Roche, Germany), for 30 min, and the lysates were centrifuged (13,000×*g*) at 4 °C for 15 min. Protein concentrations were quantified using a BCA protein assay kit (Pierce, Rockford, IL, USA). Different proteins were separated via SDS–PAGE and transferred to a PVDF membrane. After being blocked in 5% non-fat milk in TBS containing 0.1% Tween-20 for 1 h, membranes were incubated overnight with primary antibodies as follows: GFAP, Nestin (Beyotime, Shanghai, China), S100B (Proteintech, IL, USA), Vimentin (Proteintech, IL, USA), PCNA (Proteintech, IL, USA), TLR4 (WanLei, Shenyang, China), CD36 (WanLei, Shenyang, China) and GAPDH (KangChen Biotech, Shanghai, China). Following washing three times, all membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, CA, USA) at room temperature for 1 h. Protein bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Pierce), and observed via an LAS-3000 imaging system (Fujifilm, LAS3000 Luminescent Image Analyze, Japan). The relative signal densities were analysed using ImageJ software (National Institutes of Health, Bethesda, MD, USA), and GAPDH density was used as the internal control.

Transendothelial Electrical Resistance and Endothelial Permeability Assay

NSCs were seeded on the Matrigel-coated reverse side of a 0.4-μm Transwell (Corning Inc., Corning, NY, USA) upper chamber at 5×10^4 cells/well in NSCs culture medium. The Transwell upper chamber was kept upside down in the 24-well plate overnight to permit NSC attachment to the reverse side. Two days later, the NSCs culture medium was replaced with astrocyte culture medium, with resistin or PBS being added. After 5 days, 1×10^5 HBMECs were planted in the upper chamber of a Transwell insert. The integrity of the HBMEC monolayer was monitored by daily transendothelial electrical resistance (TEER) measurements, using a Millicell-ERS endothelial volt-ohmmeter (World Precision Instruments Inc., Sarasota, FL, USA). The TEER values were measured at 7, 8 and 9 days after replacement of astrocyte culture medium. FITC-glucan measurement was performed as follows. The cells were thoroughly rinsed with PBS. FITC-conjugated β-glucan (Sigma-Aldrich, Darmstadt, Germany) dissolved in serum-free DMEM without phenol red was added onto the Transwell insert to give a final concentration of 0.5 mg/ml. Two hours later, the medium in the lower chamber was collected, and the FITC-conjugated β-glucan content was evaluated at 492 nm. The permeability coefficient (Pe) of FITC-dextran was calculated in centimetres/minute (cm^2/min) as described by Vandenhaute et al. [23].

Gene Chip Analysis

The cells were washed thoroughly by PBS at 4 °C and the samples were administered Trizol reagent. Whole genome expression profile analysis was performed by Shanghai Zhuo Li Biological Technology. The samples were hybridized with a mouse MOA 2.1 Chip, and the arrays were scanned using an Agilent Microarray Scanner (G2505C).

Real-time PCR

Total RNA was prepared using Trizol (Life Technologies: Carlsbad, USA) according to the manufacturer's protocol, treated with RNase-free DNase I (TaKaRa Bio Inc., Kusatsu, Japan), and reverse-transcribed with M-MLV Reverse Transcriptase (Promega, Madison, WI, USA). Real-time PCR was performed with SYBR Premix *Ex Taq* (Takara Biotechnology, Dalian, China) according to the manufacturer's protocol. Real-time PCR was performed using an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with SYBR® Selected Master Mix (ABI) in accordance with the manufacturer's protocol. cDNAs were amplified in an amplification scheme as follows: (94 °C, 3 min; 35 cycles of 94 °C, 30 s, 61 °C, 30 s and 72 °C, 30 s; 72 °C, 10 min). All primers used are listed in Table 2.

Statistical Analysis

GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) was used to perform statistical analysis. Statistical significance was assessed using two-tailed Student′s *t*-test ($\alpha = 0.05$) between two groups. One-way ANOVA was followed by the post hoc Tukey test for multiple comparisons. Differences were considered statistically significant at a p value < 0.05.

Table 2 Realtime PCR primer sequences

Name	Sequence	
	Sense (5′–3′)	Antisense (5′–3′)
CD36	CTCATGCCAGTCGGAGACAT	GCCACGTCATCTGGGTTTTG
TLR4	TCTGGGGAGGCACATCTTCT	AGGTCCAAGTTGCCGTTTCT

Results

GFAP$^+$/BrdU$^+$ Cells Decreased in Resistin Intracerebroventricular-Injected Mice

To test the effect of resistin on astrocyte proliferation, we intracerebroventricular injected BrdU with PBS or resistin in C57 mice. The results showed that there were more GFAP/BrdU double-staining positive cells in the subventricular zone of the mice without resistin injection. However, no GFAP/BrdU double-positive cells were detected in the subventricular zone of resistin-injected mice. This indicated that resistin might inhibit astrocyte differentiation (Fig. 1).

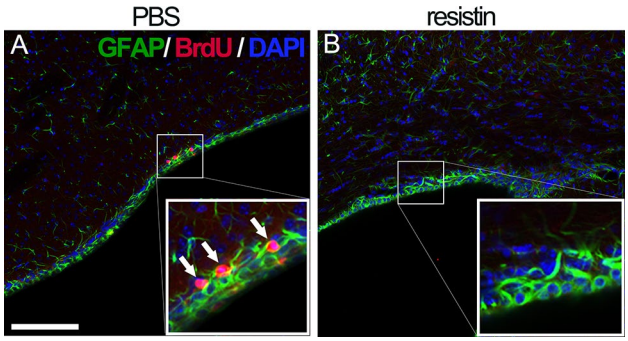

Fig. 1 Immunohistofluorescence staining of injected NSCs differentiation in hippocampus. BrdU with PBS (**a**) or resistin (**b**) were injected into 8-week mouse brain lateral ventricle (AP, ±0.34 mm; ML, ±1 mm; DV, ±2.25 mm). Vibratome slices (50 μm thick) of the corpus striatums from the PBS and resistin groups were stained with GFAP (green) and DAPI (blue) at 21 days after the injection of BrdU. The figure in lower right corner is an enlargement image of the region indicated by the white box in larger figure. The GFAP$^+$/BrdU$^+$ astrocytes are indicated by the white arrows. Scale bar = 100 μm. (Color figure online)

Resistin Reduced the GFAP/GFP Double-Positive Cell Number in the NSC-Derived Astrocyte Culture System

To test whether the decrease in GFAP/GFP double-positive cell number was due to a decrease in the proliferation of transplanted NSCs, we treated NSCs with different concentrations of resistin first. The results of cell proliferation and cytotoxicity assays showed no significant difference between controls and resistin-treated cells (Supplemental data 1A). Consistent with this result, the cell cycle assays for flow cytometry also showed no difference between the two groups (Supplemental data 1B, C). This suggested that resistin alone does not affect the proliferation of NSCs in NSC culture medium.

To investigate the effect of resistin on NSC differentiation, we removed EGF and bFGF from the NSC culture medium and used serum to simulate NSC differentiation. The proliferation state was first assayed in the differentiated system. The flow cytometry results showed that the S phase cell numbers increased significantly after 24 or 72 h resistin (10, 100 ng/ml) incubation, greater than the controls (Fig. 2a, b). This indicated that in the differentiated state, resistin inhibited cell proliferation. It is necessary to investigate whether resistin further influences cell differentiation.

Resistin Influence on NSCs differentiation

GFAP is a type III intermediate filament protein and helps maintain astrocyte mechanical strength [24]. Nestin is a type VI intermediate filament (IF) protein [25] that plays a role

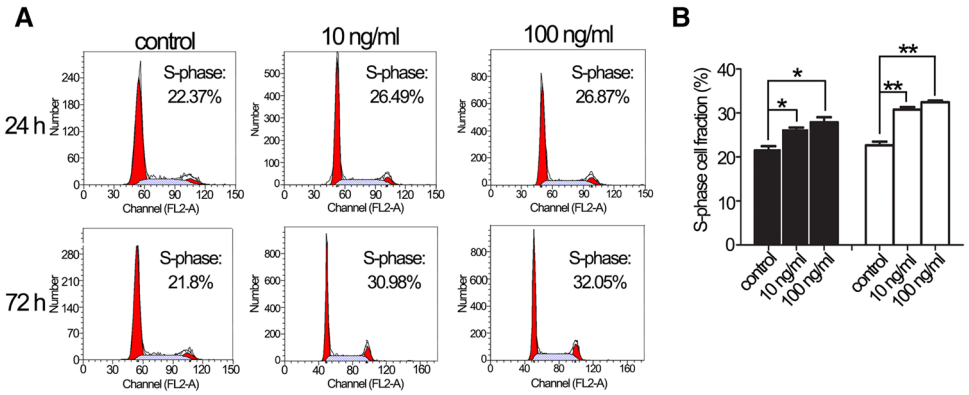

Fig. 2 Effect of resistin on NSC cell cycle. **a** Effect of resistin on NSC cell cycle during spontaneous differentiation. NSCs were incubated with resistin (10 ng/ml or 100 ng/ml) for 24 or 72 h, respectively, then flow cytometry cell cycle analysis was performed. **b** Statistical analysis of S phase cell fraction. The results represent three independent experiments. One-way ANOVA was performed to compare resistin-treated groups and controls. Data are shown as the means ± standard deviations (SD). *$p < 0.05$ versus control. **$p < 0.01$ versus control

in regulation of the assembly and disassembly of intermediate filaments and participates in cell remodelling [26]. Even though its exact function remains poorly understood, GFAP is extensively used as an astrocyte marker and nestin is regarded as an NSC marker. We performed a differentiation assay in both a unidirectional and a directional differentiation (astrocyte) culture system and then observed the GFAP expression. The immunofluorescence results showed that in the unidirectional culture system, the number of GFAP-positive cells significantly decreased (Supplemental data 2A). However, the number of nestin-positive cells increased (Supplemental data 2A). Proliferating cell nuclear antigen (PCNA) acts as a processivity factor of DNA polymerase δ [27] and is thought to be related to cell proliferation. Inconsistent with the NSC proliferation assay, PCNA expression significantly decreased (Supplemental data 2B, C) in both 10 and 100 ng/ml resistin-incubated cells at the indicated time points (day 3 and day 7).

Meanwhile, the effect of resistin on astrocyte differentiation was investigated in the committed NSCs differentiation system. On the 7th day after astrocyte differentiation, the number of differentiated astrocytes in the resistin-treated group was significantly less than that of the cells without resistin treatment (Fig. 3a). The percentage of undifferentiated cells significantly increased with the treatment of resistin (Fig. 3b). S100B is a Ca^{2+}-binding protein produced mainly by astrocytes. A prominent role of this protein appears to be the promotion of vascular inflammatory responses through interaction with the receptor for advanced glycation end products (RAGE) [28]. Both nestin and vimentin are currently widely used as neural progenitor cell (NPC) and NSC markers [29, 30].

The results of western blot showed that after 7 days of resistin treatment, the GFAP and S100B expression in 100 ng/ml resistin-treated cells decreased significantly, while the expression of nestin and vimentin increased (Fig. 3c, d). With the treatment of different dose resistin, the number of S100β-positive cells decreased, however, the number of nestin-positive cell increased (Fig. 3e, f). The above results suggested that resistin inhibits astrocyte differentiation and maintains NSCs undifferentiated.

Resistin-Inhibited Astrocyte Differentiation is Mediated Through TLR4

To investigate the mechanism underlying resistin inhibiting astrocyte differentiation, a microarray study of resistin-treated NSCs was performed. It showed that CD36 expression at the RNA level was upregulated in resistin-treated NSCs (Supplemental data 3A). CD36 is a type II clearance receptor involved in proinflammatory responses [31–33]. In a cerebral haemorrhage rat model, it was found that the level of CD36 expression is negatively regulated by inflammatory factors [34]. The inflammatory reaction downregulates the expression of CD36 protein and influences the haematoma phagocytosis and clearance [35, 36]. In addition, CD36 may be involved in the formation of astrocytes following ischaemia [37]. To further verify the expression level, protein expression of CD36 was assayed using western blotting (Supplemental data 3B). This showed that resistin increased the protein expression of CD36 in differentiated astrocytes.

Resistin plays role in influencing the activity of target cells through the Toll-like receptor 4 (TLR4) [38, 39]. To further confirm the role of TLR4 in resistin-inhibited astrocyte differentiation, TLR4 in NSCs was interfered with by siRNA, with approximately 80% of cells successfully transfected (Supplemental data 4). This showed that TLR4 expression at the mRNA and protein levels was downregulated significantly in siTLR4-interfered NSCs (Fig. 4a, b). The resistin-induced decrease of GFAP-positive cell number was rescued significantly in siTLR4-pretreated cells (Fig. 4c).

Studies have shown that the TLR4 signalling pathway can downregulate the expression of CD36 in monocytes when monocytes phagocytose neutrophils via auto-apoptosis [9]. In $TLR4^{-/-}$ intracerebral haemorrhagic mice, the expression of CD36 around the haematoma is upregulated [40]. This indicates that CD36 expression may be regulated by TLR4.

To refine the mechanism of interaction between TLR4 and CD36, effective TLR4 siRNA and CD36 siRNA were used to transfect NSCs. The astrocyte culture medium was administered 24 h later and the expression of TLR4 and CD36 was detected by observing mRNA and protein levels. This showed that resistin-induced upregulation of CD36 expression was inhibited by TLR4 interference (Fig. 5a, b). However, CD36 interference did not influence resistin-induced TLR4 increase (Fig. 5c, d). These results indicated that resistin affects the expression of CD36 by mediation of TLR4, which might be the receptor of resistin on astrocytes.

Resistin Increases Blood–Brain Barrier Permeability by Inhibiting Astrocyte Differentiation In Vitro

The blood–brain barrier (BBB) is a highly specialized structural and biochemical barrier regulating the entry of blood-borne molecules into the brain and preserving ionic homeostasis within the brain microenvironment [41]. Disruption of the BBB is an early feature of lesion formation that correlates with clinical exacerbation, leading to oedema, excitotoxicity, and entry of serum proteins and inflammatory cells. The activity of astrocytes is related to BBB integrity [42]. The restrictive nature of BBB is due to tight junctions between adjacent endothelial cells at the apical membrane, resulting in high TEER and low paracellular permeability [41]. To investigate whether resistin influences BBB integrity by modulating astrocyte differentiation, we established

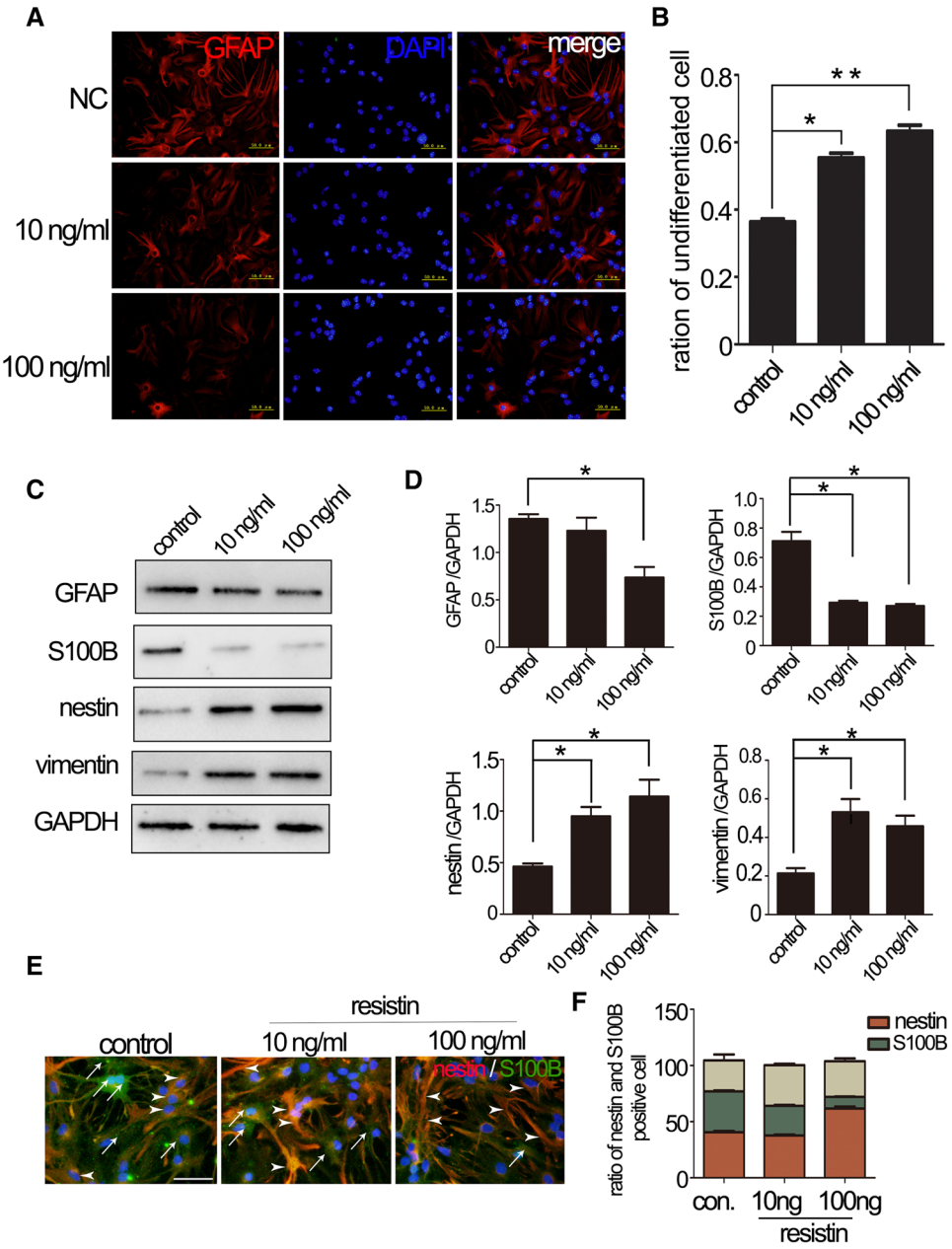

Fig. 3 Expression of astrocyte markers during NSCs committed differentiation. **a** NSCs were treated with resistin at 10 ng/ml and 100 ng/ml, respectively. 7 days later, GFAP expression was visualized by immunofluorescence (red). DAPI was used to visualize cell nuclei (blue). Scale bar: 50 μm. **b** The GFAP-negative cell fraction was calculated. GFAP-negative cell fraction = (total cell number − positive cell number)/total cell number. **c** The expression of the NSC differentiation markers GFAP, S100B, nestin and vimentin were determined by Western blot. **d** Quantification showing GFAP, S100B, nestin and vimentin expression. The results represent three independent experiments. One-way ANOVA was performed to compare resistin-treated groups and controls. Data are shown as the means ± standard deviations (SD).*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$ versus control. **e** The expression of S100B and nestin on resistin treated cells. Cells were stained with S100B (green), nestin (red) and DAPI (blue). Arrow indicated S100BS positive cells. Arrow head indicated nestin positive cells. Scale bar: 50 μm. **f** Statistical analysis of S100B or nestin-positive cell fraction in total cells. (Color figure online)

a BBB model in vitro by co-culture of human brain microvascular endothelial cells (HBMECs) and NSCs-derived astrocytes (Fig. 6a). Brain microvascular endothelial cells serve as a readily available in vitro model for studying the properties of the human BBB [43]. Resistin treatment significantly decreased TEER (Fig. 6b) and increased permeability of HBMEC (Fig. 6c) in the HBMEC-astrocyte coculture group. In order to know whether HBMEC work well in the system, we analyzed the permeability difference between astrocyte with or without HBMEC. It showed that the permeability was different (Fig. 6c). It indicated that HBMEC in the system was working properly. All these results indicated that resistin influenced BBB permeability of astrocytes. The mechanism underlying resistin-inhibited astrocyte differentiation needs to be further studied.

Discussion

Different CNS diseases and trauma cause activation of the innate and adaptive immune responses that influence the NSCs [44]. Resistin is a hormone that was found being produced and released from adipose tissue to serve endocrine functions involved in insulin resistance in mice [45]. Recently, resistin was found to be present in human cerebral spinal fluid [46, 47] in various neurological diseases and to

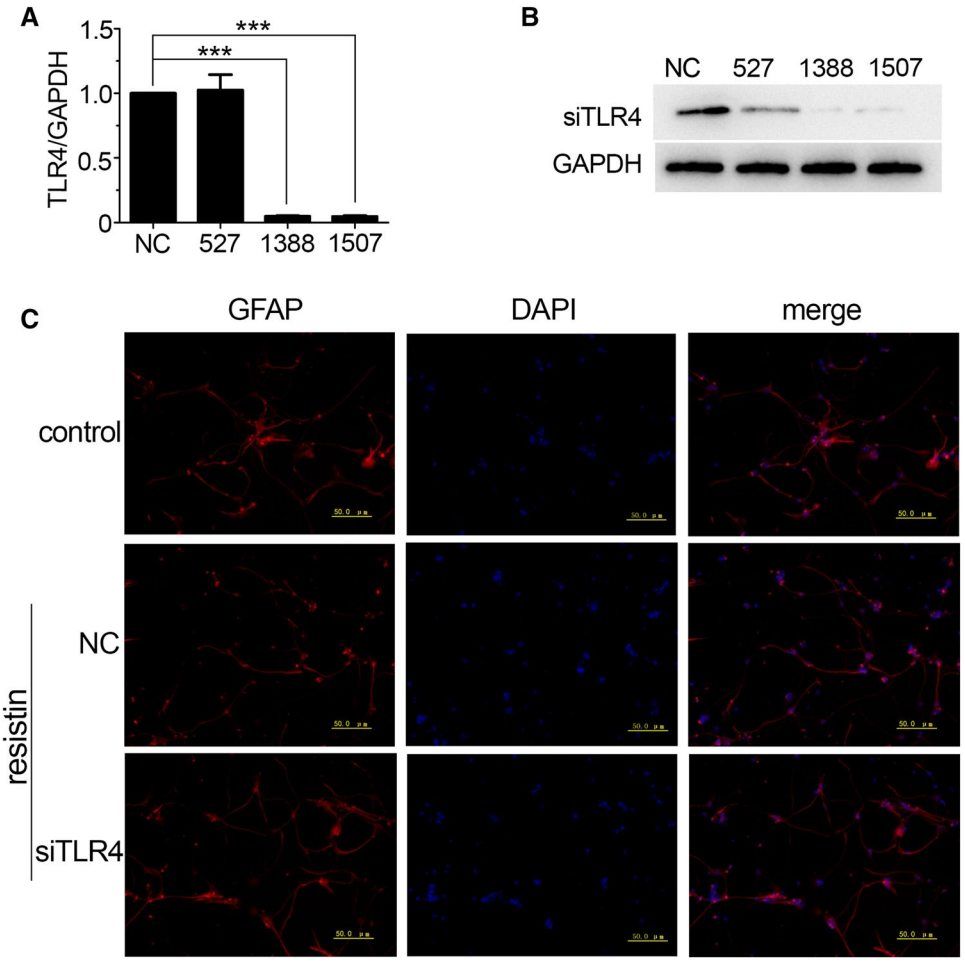

Fig. 4 Effect of TLR4 interference on NSC differentiation. NSCs were transfected with siTLR4, and the expression of TLR4 expression was detected by Real-time PCR (**a**) and Western blot (**b**). **c** Expression of GFAP in TLR4-siRNA interfered NSCs. TLR4-siRNA was added to resistin (100 ng/ml)-incubated NSCs for 7 days. GFAP expression was visualized by immunofluorescence (red). DAPI was used to visualize cell nuclei (blue). Scale bar: 50 μm. (Color figure online)

affect the function of neural tissue cells [48]. As an inflammatory factor, resistin participates in influencing proinflammatory cytokine release from human adipocytes, potentially via the integration of nuclear factor-κB and JNK signalling pathways [49]. Thus, it is necessary to investigate the role resistin plays in the brain.

Neuroinflammation has been demonstrated to affect important processes in the brain, such as adult neurogenesis. The initial pathological damage, such as massive protein aggregation, leads to a persistent inflammatory/cytotoxic reaction while enlarging the secondary neuroinflammatory reaction, leading to altered neurological function and neurological deficits in the corresponding area of dementia [3, 4]. Effects of different inflammatory factors on neurogenesis are dissimilar. For this reason, many therapeutic approaches have been developed to avoid or mitigate the deleterious effects caused by the chronic activation of the immune response [50]. Astrocytes form borders (glia limitans) that separate neural from non-neural tissue along perivascular spaces, meninges and tissue lesions in the central nervous system (CNS) [50]. Astrocytes are emerging as pivotal regulators of CNS inflammatory responses in restricting neurotoxic inflammation, such as restricting leukocyte trafficking in the CNS, releasing proinflammatory factors, and anti-inflammation by forming astrogliosis and scar tissue [51]. In the present study, we found that resistin inhibited astrocyte GFAP expression. To determine whether the inhibition of GFAP expression reflected a differentiation arrest rather than aberrant marker expression, we examined the expression of nestin and vimentin. The experiments showed that resistin-treated NSC-derived-astrocytes maintained nestin and vimentin expression, which represented an astrocyte differentiation arrest. However, resistin does not influence the proliferation of neural stem cells. The role of resistin on astrocytes has not been previously reported, and it is therefore necessary to investigate the function of resistin-induced astrocyte differentiation inhibition.

The BBB is mainly composed of brain microvascular endothelial cells (ECs), astrocytic endfeet, pericytes and the basement membrane (BM) [52]. BBB has a series of additional properties in tightly regulating the movement of molecules, ions, and cells between the blood and the CNS [53]. BBB dysfunction can lead to ion dysregulation and altered signalling homeostasis, as well as the entry of immune cells

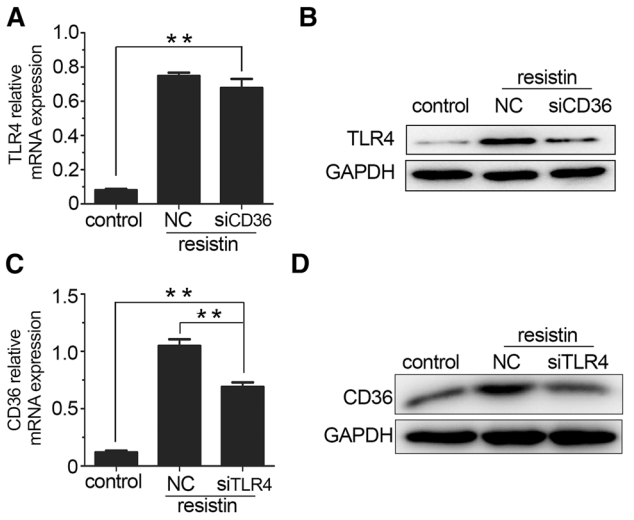

Fig. 5 Interference effect of TLR4 or CD36 on expression of CD36 or TLR4. NSCs were treated with resistin at 10 ng/ml or 100 ng/ml for 7 days. TLR4 expression was detected by Real-time PCR (**a**) and Western blot (**b**) in CD36-interfered NSCs. One-way ANOVA was used for repeated measurements. $^{**}p<0.01$ versus control. CD36 expression was detected by real-time PCR (**c**) and western blot (**d**) in TLR4-interfered NSCs. One-way ANOVA was used for repeated measurements. $^{**}p<0.01$ versus control or NC. All the experiments were independently performed three times (n = 3)

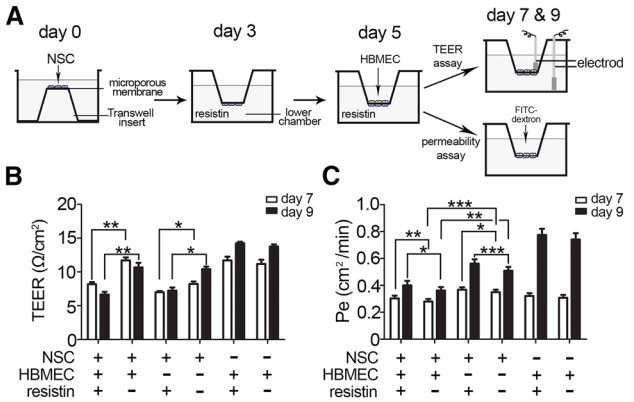

Fig. 6 The integrity and permeability of BBB. **a** Schematic diagram of in vitro BBB model. The NSCs cultured on the reverse side of Transwell insert were placed on a 24-well plate. Resistin (100 ng/ml) was present in the lower chamber after NSCs attached to the membrane of the insert for 9 days. HBMECs were plated in 24-well fibronectin-coated Transwell insert plates (pore size: 0.4 mm) on 5th day and grown for 4 days. Time-dependent changes in the TEER and permeability of the BBB were subsequently measured. **b** Time-dependent changes in TEER. **c** Effect of resistin on BBB permeability. P_e was calculated using the surface area of the Transwell insert (0.4 μm pore size, membrane surface 4.2 cm^2) to divide the permeability value for the endothelial monolayer and astrocyte co-culture. One-way ANOVA was used for repeated measurements. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ versus control, the experiment was independently performed three times (n = 3)

and molecules into the CNS, processes that lead to neuronal dysfunction and degeneration. Our previous studies showed increased mononuclear cell transendothelial migration into the brain of APP/PS1 Alzheimer's disease mice [54]. In addition to ECs, astrocytes are emerging as pivotal regulators of endothelial blood–brain barrier properties that can, via specific molecular mechanisms, either open or maintain barrier functions, and do so in a context-dependent manner as regulated by specific signalling events [51]. This suggests that astrocytes may cause monocytes to enter the brain by altering BBB permeability. It has been reported that resistin promoted the recruitment of inflammatory monocytes and the establishment of an innate proinflammatory cytokine environment in multiple helminth infections [55]. Resistin exhibits mitogenic properties towards human endothelial [56, 57] and smooth muscle cells [58] by reducing eNOS expression, potentiating the expression of adhesion molecules (ICAM, VCAM, ET-1, PAI-1) [59]. These results indicated that resistin was able to promote BBB disruption. Using the in vitro Transwell co-culture system containing HBMEC and mNSCs-derived astrocytes, we loaded resistin in the lower chamber of a Transwell plate mimicking the BBB microenvironment and found that resistin did not change the TEER or permeability of HBMEC. However, resistin significantly decreased the TEER and increased permeability of HBMEC in the HBMEC-astrocyte coculture group. The astrocyte structural proteins GFAP and vimentin [60] are required for astrocyte scar formation [61] and their absence markedly exacerbates the inflammation and tissue pathologies associated with autoimmune attack [62], stroke [63, 64] and neurodegeneration due either to lipid storage defects [65] or amyloid-beta accumulation [66]. This suggested that intracerebral resistin increased BBB permeability by inhibiting astrocyte differentiation, and increased resistin expression could be predictive of exacerbation of BBB disruption. These data indicated that resistin might increase influence of myeloid-derived factors on neural cells through weakened astrocyte function. These results also provide an experimental basis for the effect of resistin on the activity of neural stem cells and help us better understand the pathological process of inflammation in brain diseases.

TLR4 is an important molecule that has been proposed to contribute to resistin-induced effects on target cell [38, 39, 67]. The TLR4 and CD36 scavenger receptor belong to different membrane receptor families. Studies have shown that TLR4 signalling can downregulate the expression of CD36 in monocytes, when they engulf autologous apoptotic neutrophils [68]. The expression of CD36 around the haematoma is upregulated in $TLR4^{-/-}$ mice after intracerebral haemorrhage [69]. These data showed that resistin induced inflammation through mediation of TLR4. In the present study, we found that resistin inhibited NSC-derived astrocyte differentiation through TLR4. Resistin-induced

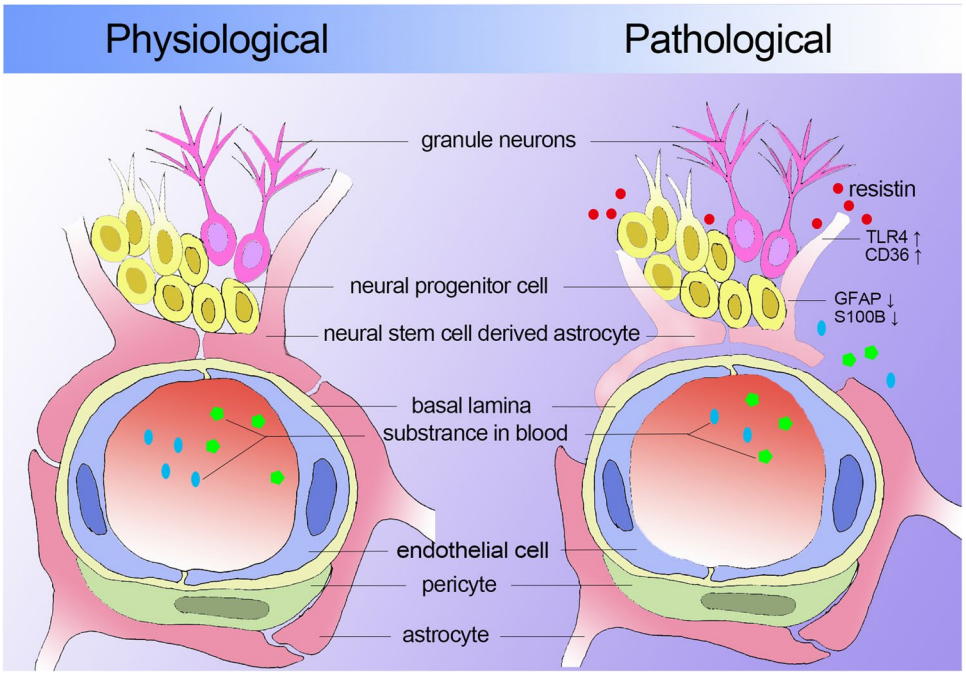

Fig. 7 Graphical model of the role which resistin playing on neural stem cells. Resistin inhibits astrocyte differentiation via mediation of TLR4 and CD36 in pathological condition. The inhibition of astrocyte differentiation leads to the increase of BBB permeability

upregulation of CD36 expression was inhibited by TLR4 interference. However, CD36 interference did not influence resistin-induced TLR4 increased expression. The detailed mechanism underlying TLR4-regulated CD36 expression in astrocyte differentiation needs to be further investigated. These results indicate that resistin affects the expression of CD36 by mediation of TLR4 on astrocytes. However, these results are contrary to that of TLR4 negative regulation of CD36 on inflammatory cells. We think the reason is that CD36 and TLR4 molecules regulation might be different in various cells and diseases. In our study, we detected different CD36 and TLR4 expression mode. First, previous reports about CD36/TLR4 were mainly focus on monocyte or macrophage. NSCs are different to monocytes or macrophages. The pathways in inflammation cells cannot be fully copied in NSCs. Second, the signal pathways involved in differentiation are not the same as inflammation. Our data might provide new functions of TLR-4/CD36 on astrocyte differentiation.

However, there are a few limitations to the present study. Lee et al. reported that adenylyl cyclase-associated protein 1 (CAP1) is a receptor for human resistin and mediates inflammatory actions of human monocytes [70]. Onuma et al. reported that plasma resistin is associated with single nucleotide polymorphisms of decorin gene in the general Japanese population. It is possible that human decorin is a human resistin receptor [71]. In the present study, we verified that resistin played role on target cell through TLR4, however, the expression of the reported resistin receptors on mice cell has not been tested. It is necessary to further identify resistin receptors on mouse neural stem cells in future studies.

In conclusion, our study revealed that resistin inhibits astrocyte differentiation via mediation of TLR4 and CD36 in pathological condition. The inhibition of astrocyte differentiation leads to the increase of BBB permeability (Fig. 7). These findings may provide useful clues to understanding the mechanisms underlying chronic inflammation activation in neurological diseases.

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References

1. Członkowska A, Kurkowska-Jastrzębska I (2011) Inflammation and gliosis in neurological diseases–clinical implications. J Neuroimmunol 231(1–2):78–85
2. McGeer PL, Itagaki S, McGeer EG (1988) Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. Acta Neuropathol 76(6):550–557
3. Imamura K, Hishikawa N, Ono K, Suzuki H, Sawada M, Nagatsu T, Yoshida M, Hashizume Y (2005) Cytokine production of activated microglia and decrease in neurotrophic factors of neurons in the hippocampus of Lewy body disease brains. Acta Neuropathol 109(2):141–150
4. Mrak RE, Griffin WS (2007) Common inflammatory mechanisms in Lewy body disease and Alzheimer disease. J Neuropathol Exp Neurol 66(8):683–686

5. Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, Karlström H, Lendahl U, Frisén J (2000) Generalized potential of adult neural stem cells. Science 288(5471):1660–1663
6. Altman J, Das GD (1965) Post-natal origin of microneurones in the rat brain. Nature 207(5000):953–956
7. Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137(4):433–457
8. Kempermann G, van Praag H, Gage FH (2000) Activity-dependent regulation of neuronal plasticity and self repair. Prog Brain Res 127:35–48
9. Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH (2003) Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development 130(2):391–399
10. Seidenfaden R, Desoeuvre A, Bosio A, Virard I, Cremer H (2006) Glial conversion of SVZ-derived committed neuronal precursors after ectopic grafting into the adult brain. Mol Cell Neurosci 32(1–2):187–198
11. Palmer TD, Willhoite AR, Gage FH (2000) Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 425(4):479–494
12. Oatley JM, Brinster RL (2012) The germline stem cell niche unit in mammalian testes. Physiol Rev 92(2):577–595
13. Chen S, Lewallen M, Xie T (2013) Adhesion in the stem cell niche: biological roles and regulation. Development 140(2):255–265
14. Ghiaur G, Yegnasubramanian S, Perkins B, Gucwa JL, Gerber JM, Jones RJ (2013) Regulation of human hematopoietic stem cell self-renewal by the microenvironment's control of retinoic acid signaling. Proc Natl Acad Sci USA 110(40):16121–16126
15. Mirshekar-Syahkal B, Fitch SR, Ottersbach K (2014) Concise review: from greenhouse to garden: the changing soil of the hematopoietic stem cell microenvironment during development. Stem Cells 32(7):1691–1700
16. Jung HS, Park KH, Cho YM, Chung SS, Cho HJ, Cho SY, Kim SJ, Kim SY, Lee HK, Park KS (2006) Resistin is secreted from macrophages in atheromas and promotes atherosclerosis. Cardiovasc Res 69(1):76–85
17. Kusminski CM, McTernan PG, Kumar S (2005) Role of resistin in obesity, insulin resistance and Type II diabetes. Clin Sci (London) 109(3):243–256
18. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A (2005) Resistin, an adipokine with potent proinflammatory properties. J Immunol 174(9):5789–5795
19. Demirci S, Aynalı A, Demirci K, Demirci S, Arıdoğan BC (2017) The serum levels of resistin and its relationship with other proinflammatory cytokines in patients with Alzheimer's Disease. Clin Psychopharmacol Neurosci 15(1):59–63
20. Qi Y, Jia K, Zhang DQ, Li T, Li LM, Zhang LJ, Wang J, Gao CL, Sun LS, Shi FD, Yang L (2016) Increased resistin levels in the serum and cerebrospinal fluid of patients with neuromyelitis optica. Clin Chim Acta 456:176–179
21. Stins MF, Gilles F, Kim KS (1997) Selective expression of adhesion molecules on human brain microvascular endothelial cells. J Neuroimmunol 76(1–2):81–90
22. Zhang H, Zhang S, Zhang J, Liu D, Wei J, Fang W, Zhao W, Chen Y, Shang D (2018) ZO-1 expression is suppressed by GM-CSF via miR-96/ERG in brain microvascular endothelial cells. J Cereb Blood Flow Metab 38(5):809–822
23. Vandenhaute E, Culot M, Gosselet F, Dehouck L, Godfraind C, Franck M, Plouët J, Cecchelli R, Dehouck MP, Ruchoux MM (2012) Brain pericytes from stress-susceptible pigs increase blood-brain barrier permeability in vitro. Fluids Barriers CNS 9(1):11
24. Cullen DK, Simon CM, LaPlaca MC (2007) Strain rate-dependent induction of reactive astrogliosis and cell death in three-dimensional neuronal-astrocytic co-cultures. Brain Res 1158:103–115
25. Guérette D, Khan PA, Savard PE, Vincent M (2007) Molecular evolution of type VI intermediate filament proteins. BMC Evol Biol 7:164
26. Michalczyk K, Ziman M (2005) Nestin structure and predicted function in cellular cytoskeletal organisation. Histol Histopathol 20(2):665–671
27. Bravo R, Frank R, Blundell PA, Macdonald-Bravo H (1987) Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. Nature 326(6112):515–517
28. Yan SS, Wu ZY, Zhang HP, Furtado G, Chen X, Yan SF, Schmidt AM, Brown C, Stern A, LaFaille J, Chess L, Stern DM, Jiang H (2003) Suppression of experimental autoimmune encephalomyelitis by selective blockade of encephalitogenic T-cell infiltration of the central nervous system. Nat Med 9(3):287–293
29. Walder S, Ferretti P (2004) Distinct neural precursors in the developing human spinal cord. Int J Dev Biol 48(7):671–674
30. Gilyarov AV (2008) Nestin in central nervous system cells. Neurosci Behav Physiol 38(2):165–169
31. Janabi M, Yamashita S, Hirano K, Sakai N, Hiraoka H, Matsumoto K, Zhang Z, Nozaki S, Matsuzawa Y (2000) Oxidized LDL-induced NF-kappa B activation and subsequent expression of proinflammatory genes are defective in monocyte-derived macrophages from CD36-deficient patients. Arterioscler Thromb Vasc Biol 20(8):1953–1960
32. McGilvray ID, Serghides L, Kapus A, Rotstein OD, Kain KC (2000) Nonopsonic monocyte/macrophage phagocytosis of Plasmodium falciparum-parasitized erythrocytes: a role for CD36 in malarial clearance. Blood 96(9):3231–3240
33. Bamberger ME, Harris ME, McDonald DR, Husemann J, Landreth GE (2003) A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. J Neurosci 23(7):2665–2674
34. Amiel E, Alonso A, Uematsu S, Akira S, Poynter ME, Berwin B (2009) Pivotal advance: toll-like receptor regulation of scavenger receptor-A-mediated phagocytosis. J Leukoc Biol 85(4):595–605
35. Fang H, Chen J, Lin S, Wang P, Wang Y, Xiong X, Yang Q (2014) CD36-mediated hematoma absorption following intracerebral hemorrhage: negative regulation by TLR4 signaling. J Immunol 192(12):5984–5992
36. Zheng Z, Ai J, Li XA (2014) Scavenger receptor class B type I and immune dysfunctions. Curr Opin Endocrinol Diabetes Obes 21(2):121–128
37. Bao Y, Qin L, Kim E, Bhosle S, Guo H, Febbraio M, Haskew-Layton RE, Ratan R, Cho S (2012) CD36 is involved in astrocyte activation and astroglial scar formation. J Cereb Blood Flow Metab 32(8):1567–1577
38. Gan AM, Butoi ED, Manea A, Simion V, Stan D, Parvulescu MM, Calin M, Manduteanu I, Simionescu M (2013) Inflammatory effects of resistin on human smooth muscle cells: up-regulation of fractalkine and its receptor, CX3CR1 expression by TLR4 and Gi-protein pathways. Cell Tissue Res 351(1):161–174
39. Hsieh YY, Shen CH, Huang WS, Chin CC, Kuo YH, Hsieh MC, Yu HR, Chang TS, Lin TH, Chiu YW, Chen CN, Kuo HC, Tung SY (2014) Resistin-induced stromal cell-derived factor-1 expression through Toll-like receptor 4 and activation of p38 MAPK/NFκB signaling pathway in gastric cancer cells. J Biomed Sci 21:59
40. Stefanova N, Reindl M, Neumann M, Kahle PJ, Poewe W, Wenning GK (2007) Microglial activation mediates neurodegeneration related to oligodendroglial alpha-synucleinopathy: implications for multiple system atrophy. Mov Disord 22(15):2196–2203
41. Stamatovic SM, Keep RF, Andjelkovic AV (2008) Brain endothelial cell-cell junctions: how to "open" the blood brain barrier. Curr Neuropharmacol 6(3):179–192

42. Argaw AT, Asp L, Zhang J, Navrazhina K, Pham T, Mariani JN, Mahase S, Dutta DJ, Seto J, Kramer EG, Ferrara N, Sofroniew MV, John GR (2012) Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. J Clin Invest 122(7):2454–2468
43. Muruganandam A, Herx LM, Monette R, Durkin JP, Stanimirovic DB (1997) Development of immortalized human cerebromicrovascular endothelial cell line as an in vitro model of the human blood-brain barrier. FASEB J 11(13):1187–1197
44. Covacu R, Brundin L (2015) Effects of neuroinflammation on neural stem cells. Neuroscientist 23:37–39
45. Al-Suhaimi EA, Shehzad A (2013) Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity. Eur J Med Res 18:12
46. Kos K, Harte AL, da Silva NF, Tonchev A, Chaldakov G, James S, Snead DR, Hoggart B, O'Hare JP, McTernan PG, Kumar S (2007) Adiponectin and resistin in human cerebrospinal fluid and expression of adiponectin receptors in the human hypothalamus. J Clin Endocrinol Metab 92(3):1129–1136
47. Berghoff M, Hochberg A, Schmid A, Schlegel J, Karrasch T, Kaps M, Schäffler A (2016) Quantification and regulation of the adipokines resistin and progranulin in human cerebrospinal fluid. Eur J Clin Invest 46(1):15–26
48. Alsuhaymi N, Habeeballah H, Stebbing MJ, Badoer E (2017) High fat diet decreases neuronal activation in the brain induced by resistin and leptin. Front Physiol 8:867
49. Kusminski CM, da Silva NF, Creely SJ, Fisher FM, Harte AL, Baker AR, Kumar S, McTernan PG (2007) The in vitro effects of resistin on the innate immune signaling pathway in isolated human subcutaneous adipocytes. J Clin Endocrinol Metab 92(1):270–276
50. Fuster-Matanzo A, Llorens-Martín M, Hernández F, Avila J (2013) Role of neuroinflammation in adult neurogenesis and Alzheimer disease: therapeutic approaches. Mediators Inflamm 2013:260925
51. Sofroniew MV (2015) Astrocyte barriers to neurotoxic inflammation. Nat Rev Neurosci 16(5):249–263
52. Yao Y, Chen ZL, Norris EH, Strickland S (2014) Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. Nat Commun 5:3413
53. Zlokovic BV (2008) The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 57(2):178–201
54. Shang S, Yang YM, Zhang H, Tian L, Jiang JS, Dong YB, Zhang K, Li B, Zhao WD, Fang WG, Chen YH (2016) Intracerebral GM-CSF contributes to transendothelial monocyte migration in APP/PS1 Alzheimer's disease mice. J Cereb Blood Flow Metab 36(11):1978–1991
55. Jang JC, Li J, Gambini L, Batugedara HM, Sati S, Lazar MA, Fan L, Pellecchia M, Nair MG (2017) Human resistin protects against endotoxic shock by blocking LPS-TLR4 interaction. Proc Natl Acad Sci USA 114(48):E10399–E10408
56. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, Mickle DA (2003) Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation 108(6):736–740
57. Kougias P, Chai H, Lin PH, Yao Q, Lumsden AB, Chen C (2005) Effects of adipocyte-derived cytokines on endothelial functions: implication of vascular disease. J Surg Res 126(1):121–129
58. Calabro P, Samudio I, Willerson JT, Yeh ET (2004) Resistin promotes smooth muscle cell proliferation through activation of extracellular signal-regulated kinase 1/2 and phosphatidylinositol 3-kinase pathways. Circulation 110(21):3335–3340
59. Tarkowski A, Bjersing J, Shestakov A, Bokarewa MI (2010) Resistin competes with lipopolysaccharide for binding to toll-like receptor 4. J Cell Mol Med 14(6B):1419–1431
60. Middeldorp J, Hol EM (2011) GFAP in health and disease. Prog Neurobiol 93(3):421–443
61. Pekny M, Nilsson M (2005) Astrocyte activation and reactive gliosis. Glia 50(4):427–434
62. Liedtke W, Edelmann W, Chiu FC, Kucherlapati R, Raine CS (1998) Experimental autoimmune encephalomyelitis in mice lacking glial fibrillary acidic protein is characterized by a more severe clinical course and an infiltrative central nervous system lesion. Am J Pathol 152(1):251–259
63. Li L, Lundkvist A, Andersson D, Wilhelmsson U, Nagai N, Pardo AC, Nodin C, Ståhlberg A, Aprico K, Larsson K, Yabe T, Moons L, Fotheringham A, Davies I, Carmeliet P, Schwartz JP, Pekna M, Kubista M, Blomstrand F, Maragakis N, Nilsson M, Pekny M (2008) Protective role of reactive astrocytes in brain ischemia. J Cereb Blood Flow Metab 28(3):468–481
64. Liu Z, Li Y, Cui Y, Roberts C, Lu M, Wilhelmsson U, Pekny M, Chopp M (2014) Beneficial effects of gfap/vimentin reactive astrocytes for axonal remodeling and motor behavioral recovery in mice after stroke. Glia 62(12):2022–2033
65. Macauley SL, Pekny M, Sands MS (2011) The role of attenuated astrocyte activation in infantile neuronal ceroid lipofuscinosis. J Neurosci 31(43):15575–15585
66. Kraft AW, Hu X, Yoon H, Yan P, Xiao Q, Wang Y, Gil SC, Brown J, Wilhelmsson U, Restivo JL, Cirrito JR, Holtzman DM, Kim J, Pekny M, Lee JM (2013) Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1 mice. FASEB J 27(1):187–198
67. Jiang Y, Lu L, Hu Y, Li Q, An C, Yu X, Shu L, Chen A, Niu C, Zhou L, Yang Z (2016) Resistin induces hypertension and insulin resistance in mice via a TLR4-dependent pathway. Sci Rep 6:22193
68. Zamora C, Cantó E, Nieto JC, Angels Ortiz M, Juarez C, Vidal S (2012) Functional consequences of CD36 downregulation by TLR signals. Cytokine 60(1):257–265
69. Sansing LH, Harris TH, Welsh FA, Kasner SE, Hunter CA, Kariko K (2011) Toll-like receptor 4 contributes to poor outcome after intracerebral hemorrhage. Ann Neurol 70(4):646–656
70. Lee S, Lee HC, Kwon YW, Lee SE, Cho Y, Kim J, Lee S, Kim JY, Lee J, Yang HM, Mook-Jung I, Nam KY, Chung J, Lazar MA, Kim HS (2014) Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. Cell Metab 19(3):484–497
71. Onuma H, Tabara Y, Kawamura R, Ohashi J, Nishida W, Takata Y, Ochi M, Nishimiya T, Kawamoto R, Kohara K, Miki T, Osawa H (2013) Plasma resistin is associated with single nucleotide polymorphisms of a possible resistin receptor, the decorin gene, in the general Japanese population. Diabetes 62(2):649–652